Note: Errors that affect the sense have been corrected and marked (page 63):

~~Errors are shown in Red with Strike-Through~~
Corrections are shown in Blue Underlined

Page 63: The Numerical Values were typed into the wrong column

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ALBUMIN EXTRAVASATION DURING SURGERY

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for the Degree of Doctor of Medicine

by

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ABSTRACT

This thesis is concerned with the measurement of changes in the circulation during the period of surgery. A loss of albumin from the circulation is to be expected from observations and experiments made in the postoperative period. In this thesis a sensitive measurement technique is described which allows extravasation of albumin to be studied in patients undergoing elective surgery. Radio-isotope labelled albumin and radio-isotope labelled red cells are injected; the ratio between the two is not affected by blood loss or infusion and the ratio is therefore used as a measure of albumin extravasation. To measure the relative loss of albumin from the circulation the isotopes were administered some days prior to surgery to allow distribution and equilibration of the albumin. To measure the rate of loss of albumin (capillary permeability) the isotopes were administered at the time of surgery.

The results indicate that major surgery under general anaesthesia is accompanied by a loss of albumin from the circulation, an increased capillary permeability to albumin and a fall in plasma volume that is greater than can be explained on the basis of blood loss and greater than would be expected on the basis of the reduction in red cell volume.

The results suggest that patients who receive only glucose and salt solutions intravenously during major surgery will usually suffer a contraction in their blood volumes unless unacceptably large volumes of fluid are infused.

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OBJECTIVE AND SCOPE OF THIS THESIS

During the days following major surgery there is a fall in the level of albumin circulating in the blood with an accumulation of albumin at the site of surgery. This thesis is concerned with the period of surgery itself. The investigation was conducted: to develop measurement techniques suitable for use during surgery; to discover whether there were changes in the level of circulating albumin; to discover whether these changes were the result of increased extravasation rates or of changes in the rate of synthesis and lymphatic return; to discover how early significant changes occurred; and to discover whether changes might be attributable to the effects of the anaesthetic agents.

This thesis describes the above investigation preceded by a review of albumin's role in the circulation and of the changes in fluid balance following surgery.

The thesis encompasses:

1. A review of the history of the role of albumin in the circulation.
2. A review of the history of changes in fluid balance which follow surgery.
3. A description of preliminary experiments which lead to the investigation described in this thesis.
4. A description of a method developed to measure changes in the quantity of intravascular albumin during surgery, even when there is simultaneous haemodilution or haemoconcentration.
5. A description of a method developed to measure the rate at which albumin escapes from the circulation due to capillary permeability - also applicable during surgery.
6. A report of the observed changes in the quantity of intravascular albumin occurring before and during surgery.

7. A report of the observed changes in the rate of albumin extravasation, relating these to the Changes found in 6 above.

8. A report of the observed changes in blood volume relating these changes to the changes found in 6 and 7 above.

9. A report of observations on the effect on the red cell of haemodilution.

10. A discussion of the changes found and their implications for the care of the surgical patient.

INTRODUCTION

Certain aspects of fluid balance during surgery are well understood. For example, pre-operative deprivation of fluid is customary, and blood loss is observable and commonly measured. In addition, an accepted sequel to major surgery is a reduction in the excretion of water and salt. This conservation of fluid may be valuable in combating the effects of haemorrhage but it is associated with disadvantages: oliguria makes the kidney more susceptible to acute tubular necrosis, for example following a mismatched blood transfusion or during surgery in the presence of jaundice; and the clinician managing such a patient is denied the reassuring and useful physical sign of a steady flow of urine.

Antidiuretic hormone and aldosterone are normally secreted in response to changes in volume and composition of the blood. During surgery it is possible that other factors such as fear or pain could be responsible for initiating and maintaining their secretion and hence a fluid conservation. However it is equally possible that their secretion is caused in response to changes in fluid balance, including internal redistribution. Any such internal redistribution would have to he considerable because when pre-operative dehydration and surgical haemorrhage are anticipated, quantified and corrected, such correction is frequently not accompanied by a normal flow of urine.

The theory that surgery caused **a** reduction in extracellular fluid volume has now been discredited and this will be discussed further (see History section). However, as a result of the theory, greatly increased volumes of intravenous fluid were administered during surgery and resulted in increased flows of urine. This suggested the

possibility that surgery might cause a reduction in the plasma volume and that the administration of intravenous fluid could be correcting this deficiency.

Plasma volume is largely dependent on the quantity of intravascular protein, principally albumin. A loss of albumin would tend to cause a fall in oncotic pressure but any such fall would be offset by a redistribution of salt and water; plasma volume would fall and consequently the albumin level and plasma oncotic pressure would tend to remain near their original values. The haematocrit which might reflect this loss is of little value during surgery because of changes caused by blood loss, fluid infusion and blood transfusion. Consequently, in the circumstances obtaining during surgery, relatively large losses of albumin tend to remain undetected, and changes that could indicate the loss will be concealed by, or attributed to, the therapy.

In the period following major surgery in man, a rising haematocrit is observed, and in both man and animals experiment has demonstrated the postoperative accumulation of albumin at the site of surgery. There is therefore reason to suppose both that significant albumin extravasation might be occurring during surgery and that this might be relevant to the fluid balance of patients undergoing surgery.

History

Albumin's role in the circulation has been appreciated for well over a century. In 1837 and 1838 Magendie gave a series of twenty-four lectures on blood to the College of France, and these lectures were reproduced as a weekly series by the Lancet of 1838-39 (Magendie, 1839). In these lectures he discussed the results of his experiments, many of which he performed in front of his audience or during the days between one lecture and the next. Towards the end of this series he made several references to the nature and role of serum albumin and performed a number of experiments in vitro and in vivo. Some of the experiments must have intrigued his public then, even if they seem of little relevance today; one dog fell victim to an overdose of claret which enabled him to report "the blood is of a violet hue, and holds some grumous albuminous matter in suspension" (lecture 21, p. 777). However his very considerable understanding of the way albumin acted to maintain blood volume and capillary flow was revealed in the same lecture when he stated: "It would appear that . . . . when the latter (blood) loses its albuminous ingredient in any way it becomes extravasated and is imbibed by the surrounding tissues"; when discussing the difficulty of forcing fluids such as pure water through capillaries, he stated "....that if some viscid substance were added to them, they no longer encountered any obstacle to their passage". In the next lecture (p. 824) he made it clear that albumin was a constituent not just of blood but also of "all the great accumulations of fluid produced" and that it was "thrown off" from ulcerated surfaces. In the final lecture (p.889) he returned to this subject and discussed the effects of inflammation; he described both an increase in capillary permeability and a consequent extravasation of the materials of the blood. It is interesting that Magendie appears to have attached no particular importance to these ideas. However they have been the subject of repeated investigation during the subsequent

135 years. In 1896 Starling formulated the hypothesis that "the hydrostatic pressure of blood in the capillaries" which tended to cause transudation was balanced by "the osmotic pressure of the proteids of the plasma". This hypothesis nicely explained how the plasma volume was maintained while water and small molecules could pass freely between capillary and extravascular space.

Thus, before the end of the last century, it was clearly understood that albumin had an important role in the maintenance of blood volume and that it was possible for albumin to be extravasated, e.g. by inflammation. More than 50 years ago surgical trauma was shown to be followed by changes in the blood. Alteration in the composition of plasma with a reversal in the albumin/globulin ratio was demonstrated by Löhr and Löhr (1922) to be a common change following trauma and surgery. Similar findings were reported by Cuthbertson and Tompsett (1935) and by Hoch-Ligeti et al. (1953). In 1931 Blalock demonstrated the lethal effect in dogs of prolonged pinching of the intestines and of traction on the mesentery. He attributed the shock which followed to loss of plasma.

In the few years preceding 1961 several workers had reported investigations into the changes in albumin distribution which could be demonstrated following surgery and other injuries. Fox et al. (1954) reported the migration of albumin to the site of injury following a burn. Their findings were confirmed by Birke at al. (1959), who also demonstrated that there was similar albumin extravasation at the site of surgery and that in neither case did this albumin return to the circulation. Early in 1961 Høedt-Rasmussen and Jarnum investigated and excluded the possibility that much albumin loss could be explained by losses into the intestines. This paper was complemented by another later in the same year (Jarnum, 1961) in which a loss of albumin into the peritoneal cavity was described equivalent to about 600 ml. of plasma during a three-hour gastrectomy.

With such papers already published and with similar studies to be reported in the following years, it now seems almost surprising that there should have been a search for other factors which could be affecting plasma volume and fluid balance. However a series of cases were described first by Shires et al. (1961) and again by Shires (1968) in which measurements of the extracellular fluid volume were made using a radio-sulphate dilution technique. On the basis of these observations it was suggested that surgery was accompanied by a reduction in the volume of the extracellular fluid. There was no satisfactory explanation for where the fluid might have gone and, indeed, the findings were eventually shown to originate from a faulty measurement technique (see below).

Nevertheless Shires' work started a revolution in the management of the fluid balance during surgery. For most of the next decade there was a massive increase in the quantity of fluid administered during surgery. Nearly all of this fluid was similar in composition to the extracellular fluid supposed to have been lost and was administered as lactated Ringer's solution (Hartmann's solution); patients commonly received several litres of fluid in the course of an operation lasting two or three hours. Not surprisingly, urine excretion was usually maintained; this was readily interpreted as confirmation that the Ringer's lactate infusion was correcting the deficiency in the extracellular volume.

In the year following Shires et al.'s paper, Williams et al. (1962) employed ^{131}I R.I.H.S.A. (radio-iodinated human serum albumin) to demonstrate a reduction in the plasma volume following major surgery. And in a series of investigations in animals and in man, Mouridsen and Faber (1966) and Mouridsen (1967, 1968 and 1969) showed that albumin was lost at an increased rate in the area of the wound, that the albumin tended to remain in the wound for many days and that there was an associated hypoalbuminaemia

In the year following Shires' second paper describing his findings using radio-sulphate, Roth et al. (1969) published a critical analysis of the use of large volumes of Ringer's lactate and they explained the error in Shires' method of measuring the extravascular volume. In normal people a single ten-minute sample provides a reasonable estimate of the dilution and thus of the extracellular volume. However the rate of equilibration is markedly reduced following surgery. Consequently the plasma sulphate level recorded is higher and wrongly indicates a reduced extracellular volume. By employing serial sampling and by retropolating the line of log Activity/time to the time of injection, Roth et al. demonstrated that the volume of the extra-cellular space was the same after surgery as it was before. In the same year Moss (1969) drew attention to the change which occurs in the distribution of fluid as more and more lactated Ringer's solution is given. Initially, when one litre is infused, about 1/5 of the volume (200 ml.) remains in the blood. As the volume infused rises the amount retained in the circulation falls progressively to about 1/10 or 1/20 of the amount infused. Also, in the same year, Hutchin et al. (1969) reported that the diuresis of salt and water which followed infusion of Ringer's lactate solution was obtained at the expense of risking severe pulmonary oedema. Thus, in one year, the theory that surgery might cause a reduction in the extracellular fluid volume was shown to be without an adequate foundation and, in addition, the intravenous infusion of large volumes of fluid was shown to be dangerous.

An alternative explanation of Shires' finding is that albumin extravasation accompanies surgery and causes a redistribution of fluid. This explanation would accord well with the finding postoperatively of the low plasma albumin levels reported by Löhr and Löhr (1922), by Cuthbertson and Tompsett (1935) and by Hoch-Ligeti et al. (1953) and it could explain the oliguria which follows surgery as well as the diuresis which can be obtained by infusing large volumes

of lactated Ringer's solution. It would explain the reduction in plasma volume demonstrated by Williams et al. (1962) and by Basset and Talbot (1968) and would explain the more recent demonstration by Irvin et al. (1972) that the use of a balanced salt solution can prevent the development of a plasma volume deficit as well as preventing the usual salt and water conservation. The theory that the circulation might be significantly depleted of albumin is supported by the findings of Mouridsen and Faber (1966), Chandrasekharan (1969) and Jarnum (1961). These workers all describe increased loss of albumin occurring at the site of injury.

A mechanism explaining albumin extravasation and which might allow control over permeability to albumin is provided by the demonstration by Majno and Palade.(1961) that there are reversible gaps in the walls of venules and small veins, that these gaps are influenced by histamine and serotonin and that they rather than the capillaries appear to provide a site for the extravasation of albumin. (Despite this the terms "capillary permeability and "transcapillary escape" are retained in this thesis as they are in widespread use and give rise to no ambiguities in the context used.) Within minutes of an injury to vital tissue, it is possible to demonstrate increased local levels of histamine and serotonin, a point used in forensic science to differentiate between pre- and post-mortem injuries (Raekallio, 1972). However, opposing effects might be encountered elsewhere in the body during surgery, anaesthesia and haemorrhage. Both haemorrhage (Lazarov, 1969) and plasmapheresis (Wraight, 1974) have been shown to result in a fall in capillary permeability to albumin, and such a fall might compensate for or conceivably even exceed the losses due to surgery.

Albumin is normally distributed about equally between the intra-vascular and extravascular spaces. The normal turnover of albumin between the circulation and the extravascular space involves extravasation from the venules and return via the lymphatics. The rate of loss from the circulation is estimated to be about 5% of the

intravascular albumin per hour, i.e. about 6 g/hr. or about 0.05 of the cardiac output (Yoffey and Courtice, 1970; Parving and Gyntelberg, 1973). The turnover of albumin due to synthesis and degradation is considerably smaller and is about 5 — 7% of the total albumin/day, i.e. about 0.6 g/hr. (Rothschild et al., 1969; Skillman et al., 1967).

PRELIMINARY EXPERIMENTS

Measurement was made of the changes in albumin level, globulin level, haematocrit and haemoglobin level which accompanied various surgical procedures. The effects of haemorrhage and infusion prevented any firm conclusions being drawn from the individual changes. When the albumin level was expressed as a ratio, albumin/haemoglobin, then there appeared to be loss of albumin accompanying surgery, particularly major surgery. Although the relative inaccuracy of the measurements precluded the use of this technique as a sensitive index of changes during surgery, the experience did suggest that a suitable technique required simultaneous measurement of haemoglobin and albumin levels without the necessity of separating red cells from plasma; simultaneous two-channel gamma counting of isotope-labelled albumin and haemoglobin was selected as a suitable means of achieving this.

METHOD

1. Materials

Sterile and injectable:

^{125}I R.I.H.S.A. (Radio-iodinated human serum albumin)

^{131}I - (Radio-iodinated human serum albumin)

^{51}Cr - Na_2Cr0_4

All obtained from: The Radio Chemical Centre,
Amersham,
Buckinghamshire.

Anticoagulant:

Acid citrate dextrose solution 3 mi.

Tablets:

Pot. iodide 60 mg. B.P.

Other chemicals

Na_2Cr0_4	)	Both used as carriers to
Dried human serum albumin	)	minimise uptake by glassware of isotope during preparation of standard dilutions

2. Apparatus

The gamma emissions were counted on a two-channel Wallac Decem G.T.L. 300 auto gamma spectrometer with teletype and punched paper tape output. The spectra for the three isotopes and the settings chosen to count the isotopes are described later (see Method section 11).

An Olivetti punched paper tape reader coupled to an Olivetti 602 desk top computer was used to read the output from the Wallac.

An Olivetti 602 was employed to differentiate the activity due to each isotope in the mixture and, where appropriate, to compute the regression equation - log activity/time. Details and examples of the paper tape reading program, the two-isotope analysis program and the logarithmic regression program are also described later (see Method sections 21, 22 and 23).

A Griffin digital balance was used to weigh the specimens for gamma counting. Weights were recorded to the nearest 0.1 mg. Haematocrits were measured using a Hawksley microhaematocrit reader after centrifuging specimens in a Hawksley microhaematocrit centrifuge for three minutes.

3. Subjects

Patients were selected for study from the list of patients awaiting non-urgent surgery. A preliminary explanation was given to each patient on the telephone. About half the patients approached in this way were clearly unable or reluctant to participate and were excluded from the study. Twenty-three patients expressed themselves willing to participate and were allocated in series case numbers 1-23. These patients were interviewed usually at home, and given a detailed explanation. Subsequent to this, patients 1, 4, 8 and 11 expressed some reservations about participating and were therefore excluded. Measurements are therefore reported in the remaining 19 cases.

4. Exclusions

The following were excluded from this study: women who were pregnant at the time or liable to further pregnancy, and men judged likely to want more children.

5. Precautions

To block uptake of radio-active iodine by the thyroid gland, every patient received 120 mg. potassium iodide orally at the time of the interview and 60 mg. daily for the following three weeks.

6. Anaesthetic techniques

The majority of the patients were operated on under general anaesthesia. The anaesthetic was standardised as follows:

Premedication: Papaveratum 20 mg., Hyoscine 0.4 mg.

Infusion: Lactated Ringer's solution via venous catheter.

Induction: Thiopentone 300 mg., suxamathonium 80 mg.

Intubation: Oral cuffed red rubber 8.0 mm. endotracheal tube.

Maintenance: Nitrous oxide 6 3/min., oxygen 3 l/min., tubocurarine 30 mg., pethidine 20 mg.

Ventilation: Controlled using non-rebreathing minute volume dividing ventilator.

Reversal: Atropine 1.2 mg., neostigmine 2.5 mg., oxygen 10 l/min., carbon dioxide 0.5 l/min. Ventilated until responding to commands.

Extubated after aspiration of pharynx.

This dosage of drugs was varied on occasion to suit the requirements of individual patients.

The above premedication was administered to all patients in the series and so was the anaesthetic except for patients 10, 13, 14, and 15, to whom an epidural anaesthetic was administered, and patient 21 who had a sigmoidoscopy without anaesthesia.

Patients 10 and 13 were anaesthetised with a lumbar epidural anaesthetic employing 0.5% bupivicaine 15 ml. and 25 ml. respectively with diazepam sedation. Towards the end of surgery in patient 13, pain was experienced and nitrous oxide/oxygen was administered by face mask until surgery was completed. In patients 14 and 15 a lumbar epidural anaesthetic was attempted employing 0.5% bupivicaine 25 ml. and 30 ml. respectively. In neither case did the patient obtain analgesia; both were intubated and ventilated with nitrous oxide and oxygen. Patient 14 received pancuronium, pethidine and halothane, and patient 15 received pancuronium and pethidine.

7. Recording of Data

Forms were prepared for recording patient's consent, details about the patient and the isotopes administered, details about each specimen of blood, and details of blood volume calculation. Photocopies of forms are included in the pocket at the back of this volume.

8. Measurement of Degree of Extravasation of Albumin Relative to Haemoglobin (D.E.A.R.T.H.)

Approximately five days prior to surgery 10 ml, of the patient's blood was taken and mixed with 3 ml. of acid citrate dextrose anticoagulant in a sterile container. To this was added 45 μCi of $^{51}Cr - Na_2Cr0_4$. The mixture was incubated for 30 minutes at room temperature with occasional mixing. Centrifuging at 1000 r.p.m. packed the cells at the bottom of the container and allowed the supernatant plasma containing ^{51}Cr not bound to the cells to be withdrawn and discarded. The cells were then resuspended in an equal volume isotonic saline. Centrifuging, withdrawal of plasma and reconstitution with saline was repeated twice and then to the mixture was added approximately 12 μCi of ^{125}I R.I.H.S.A. The mixture was re-injected intravenously. The days which elapsed prior to surgery permitted distribution of the ^{125}I R.I.H.S.A. throughout the body as well as allowing excretion of unbound ^{51}Cr and ^{125}I. During surgery, as well as during the time immediately preceding and following surgery, an intravenous infusion

of lactated Ringer's solution was used to maintain the patency of a long intravenous catheter inserted via a forearm or antecubital vein to the root of the neck. Before each specimen of blood was withdrawn (unless otherwise specified) the dead space of the catheter and stopcock was cleared by taking about 5 ml. of blood into a separate syringe. Care was taken to avoid dilution with infusion fluid while 3 ml. samples of blood were taken into sequestrene tubes. No tourniquet was used and no force was employed to withdraw the syringe plunger. The dead space blood was then re-injected and the syringe and cannula were flushed through. The blood samples were immediately and thoroughly mixed with the sequestrene anticoagulant to preclude clot formation.

The samples of blood were frozen to achieve haemolysis and were then rewarmed and mixed thoroughly. 2 ml. was pipetted from each sample into an unused previously weighed test tube which was reweighed after filling and then capped with parafilm. The specimens were placed in carriers previously counted to check that they were uncontaminated by ^{125}I or ^{51}Cr. Included in series with the specimens was an empty tube for estimation of background radiation and a sample of pure ^{51}Cr to allow estimation of the cross-over ratio. The settings selected for the gamma counter encompassed the main peak of activity of ^{51}Cr in Channel 1 and of ^{125}I in Channel 2. (See calibration of the gamma counter below, section 11.) The specimens were counted in series several times and the counts were analysed to allow a graph to be prepared showing changes in the radio activity from each isotope as well as changes in ratio between the two. Blood pressure, pulse, intravenous infusion volume and urine output in catheterised patients were all recorded.

9. Measurement of the Relative Albumin Transcapillary Escape rate R.A.T.E.

a) The patient's red cells were labelled with ^{51}Cr - Na_2Cr0_4 as described in the first section of Method 1. To the saline suspension of red cells was added approximately 10 μCi of ^{125}I or ^{131}I - R.I.H.S.A. After mixing, the isotope mixture was drawn up into a syringe which was then weighed. About one-fifth of the mixture was added to a measuring flask containing distilled water, carrier serum albumin 0.5 - 1.0 g. and carrier Na_2Cr0_4 0.5 - 1.0 g. The remainder of the mixture was injected intravenously into the patient. The syringe was reweighed before and after the injection so that the quantity added to the measuring flask and the quantity injected into the patient were both accurately known. 3 ml. samples were then taken from an intravenous cannula in the opposite arm into sequestrene tubes by the technique described in Method 1. The time of the initial injection and of the subsequent samples were all recorded, as was the volume of fluid infused via the intravenous cannula. Subsequently, the rate of fall in radio-iodine activity compared to radio-chromium activity was. calculated (as described in more detail below in sections 15 and 23) and this was used as a measure of the transcapillary albumin escape rate relative to haemoglobin. The blood pressure, pulse, intravenous infusion volume and urine output in catheterised patients were all recorded.

In a patient previously injected with R.I.H.S.A. and ^{51}Cr Na_2Cr0_4, a further injection was made after an interval of an hour or two. This injection was prepared to contain approximately 10 pCi of the R.I.H.S.A. not already used. As in Method 2a, part of this solution was measured by weight into a standard flask containing carrier

albumin and the remainder was injected into the patient, again being measured by weight. Again the rate of fail in activity due to radio iodine compared to radio chromium was calculated. Other recordings concerning the condition of the patient were made as in Method 2a.

c) In patient 19, where shortage of time precluded labelling of the patient's red cells, injection of ^{125}1 - R.I.H.S.A. was made as described in Method b. The rate of fall in the radio-iodine activity in this case was therefore compared with the haematocrit estimation instead of the radio-chromium activity. This procedure was repeated after an hour using ^{125}1 - R.I.H.S.A. Other recordings were again made as in Method 2a.

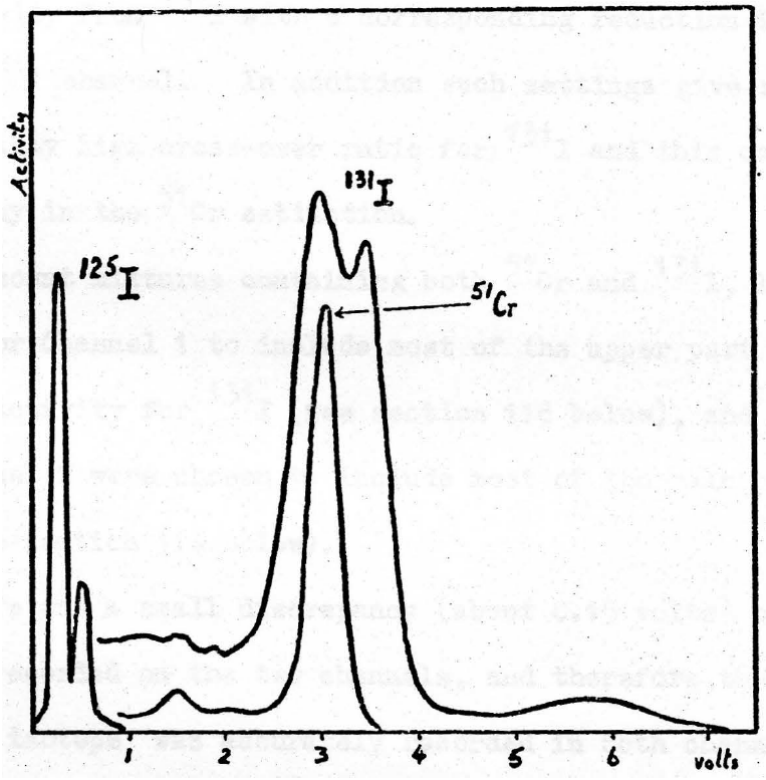
10. Measurement of Blood Volume and Whole blood Haematocrit

The technique described above (R.A.T.E., a) permitted simultaneous estimation of the apparent dilution volume (blood volume) as estimated by red cell dilution and by albumin dilution. The red cell dilution volume was multiplied by the haematocrit of the blood sample to yield the red cell volume, and the albumin dilution volume was multiplied by haematocrit) to yield the plasma volume. From these two results the blood volume and whole body haematocrit were derived.

11. Calibration of the gamma counter for counting each isotope in mixtures containing ^{51}Cr, ^{131}I and ^{125}I

a) Counting ^{125}I with either ^{51}Cr or ^{131}I

The spectrum of gamma emission for ^{125}I has its peaks of activity at energy levels below the level of the peaks for ^{131}I and ^{51}Cr:



A two-isotope mixture containing ^{125}I with either of the other isotopes was therefore counted by setting on one channel appropriate limits to count the main peaks for ^{51}Cr or ^{131}I, thus recording none of the ^{125}I activity, and on the other channel appropriate limits to count the ^{125}I activity (see section 11c below). Allowance for the small amount of activity recorded in this second channel from the first isotope was made by observing the cross-over ratio when a standard sample of isotope was counted.

11. b) Counting ^{131}I with ^{51}Cr

A mixture containing ^{51}Cr and ^{131}I cannot so satisfactorily be differentiated. Although their gamma emission spectra are different, the main peaks of activity are at the same energy level. Therefore, setting limits in each channel to include the main peak of activity does not allow separation. While limits can be chosen for ^{131}I which are entirely above the energy level of the ^{51}Cr emissions, such limits exclude about nine-tenths of the activity from ^{131}I with a corresponding reduction in accuracy in the ^{131}I channel. In addition such settings give rise to an unacceptably high cross-over ratio for ^{131}I and this causes inaccuracy in the ^{51}Cr estimation.

To count mixtures containing both ^{51}Cr and ^{131}I, limits were chosen for Channel 1 to include most of the upper part of the main peak of activity for ^{131}I (see section 11d below), and the limits for Channel 2 were chosen to include most of the main peak for ^{51}Cr (see section 11e below).

There was a small discrepancy (about 0.15 volts) between the spectra recorded on the two channels, and therefore the spectrum for each isotope was accurately recorded in both channels. The spectrum for each isotope was obtained by counting a sample repeatedly for 10 seconds at a narrow band width with an increment of 0.1 volts-between each period of counting. The graphs obtained are shown on the following pages; from the graphs appropriate settings were selected for each channel:

Channel 1 (^{131}I)	minimum 3.4	maximum 4.0
Channel 2 (^{51}Cr)	minimum 2,7	maximum 3.2

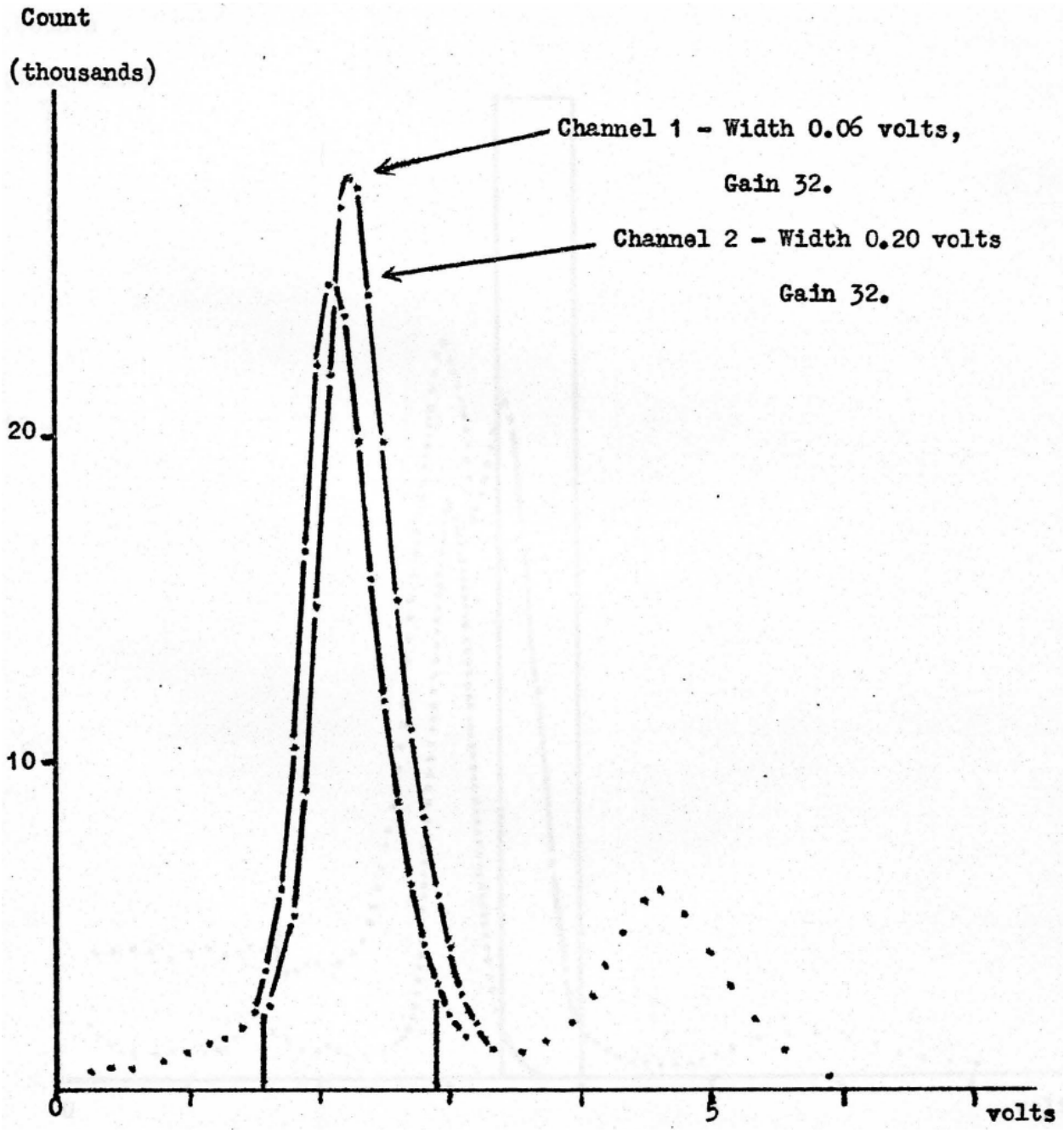
At these settings, from ^{51}Cr Channel 1 recorded about 0.06 of the activity recorded in Channel 2, and in Channel 2 the activity recorded from ^{131}I was as great as that recorded in Channel 1.

Thus in any mixture of these two isotopes a significant correction had to be applied to each channel. The magnitude of these corrections and the position of the limits in steep parts of the spectrum meant that small variations in the performance of the gamma counter might introduce considerable error. These errors were minimised by employing short periods of counting and summing the totals from several such periods. This technique tended to average out inconsistencies in the gamma counter, and in addition the short counting times reduced the error caused by isotope decay between the time that one specimen was counted and the next. It also allowed large counts to be achieved by summation and this reduced the standard error of the count. The correction for crossover, the summation of the counts and the computation, of the activity due to each isotope with the fractional standard error of the count are described in sections 12, 13, 11 and 15.

11. c) Calibration of the two-channel Wallac Decem G.T.L. 300 auto gamma spectrometer

Spectra for ^{125}I

Limits used for counting (minimum 1.6 volts
(
(maximum 2.9 volts.

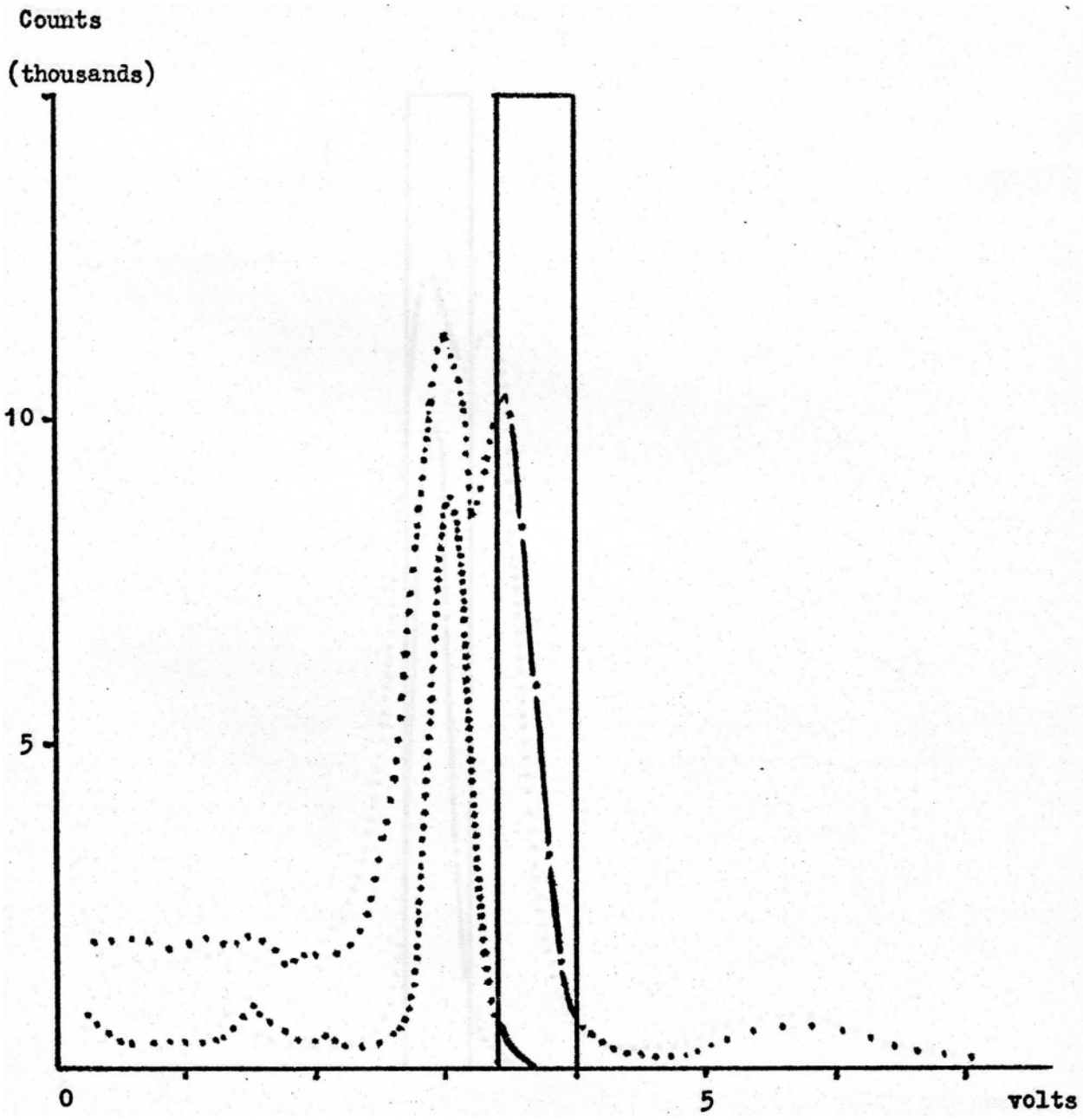


11. d) Calibration of the two-channel Wallac Decem G.T.L. 300 auto gamma spectrometer

Channel 1. Gain 4 - Width 0.06 volts

Spectra for ^{131}I and ^{51}Cr.

Vertical lines show the parts of the spectra included at the settings selected to count $^{131}1$ in a mixture with ^{51}Cr.

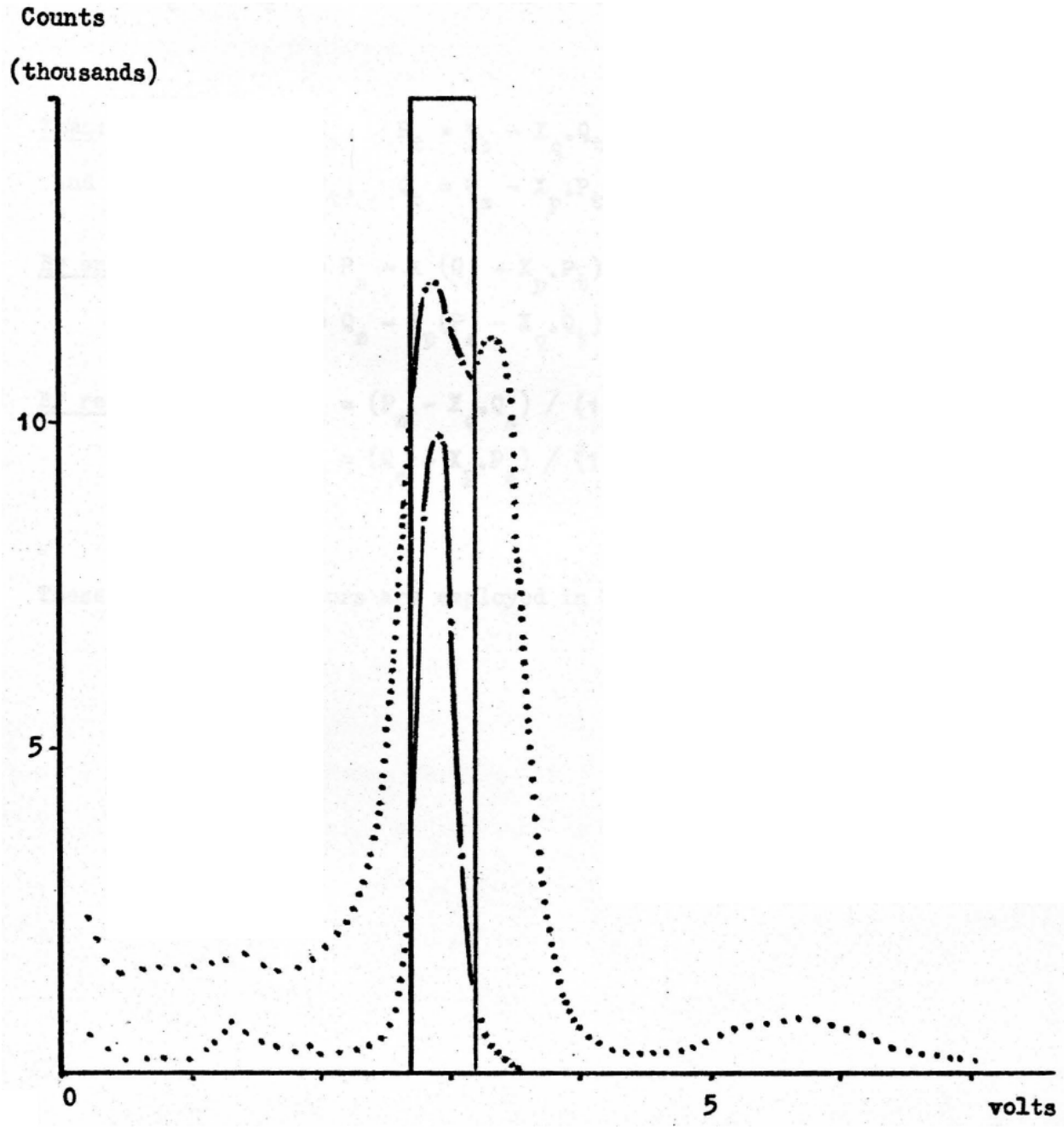


11. e) Calibration of the two-channel Wallac Decem G.T.L. 300 auto gamma spectrometer

Channel 2. Gain 4. - Width 0.20 volts

Spectra for ^{131}I and ^{51}Cr

Vertical lines show the parts of the spectra included at the settings selected to count ^{51}Cr in a mixture with ^{131}I.



12. Correction factors for cross over in a two-isotope mixture

The correction factor was derived as follows:

Where: P_s and Q_s are the counts obtained in the two channels from a mixture after correction for background,

X_p and X_q are the cross-over ratios obtained using pure isotopes;

P_t and Q_t are the counts due to each isotope (when corrected for cross over).

Then: $P_s = P_t + X_q \cdot Q_t$ therefore $P_t = P_s - X_q \cdot Q_t$

and: $Q_s = Q_t + X_P \cdot P_t$ therefore $Q_t = Q_s - X_P \cdot P_t$

By substitution:

$$P_t = P_s - X_q(Q_s - X_P \cdot P_t)$$

and $$Q_t = Q_s - X_P(P_s - X_q \cdot Q_t)$$

By rearrangement:

$$P_t = (P_s - X_q \cdot Q_s) / (1 - X_q \cdot X_p)$$

and $$Q_t = (Q_s - X_P \cdot P_s) / (1 - X_p \cdot X_q)$$

These correction factors are employed in Program B (section 22)

13. Summation of counts from the auto gamma counter

Repeated counting of each specimen minimised errors arising from several sources but resulted in an unmanageable quantity of data. A single experiment often yielded more than 400 six-digit numbers; an example of the output from an experiment is included in the pocket at the end of this volume. Manual processing of this data proved inaccurate (as well as tedious) and a program was therefore prepared to read the paper tape, which had been punched simultaneously by the teleprinter, and to summate the activity for every specimen. A description, instructions for use and annotated program are included as Program A at the end of this section (method 21).

14. Computations of activity per gram, ratios and counting error

From the summed counts for each specimen (obtained from Program A) was calculated the activity of each isotope per gram and the ratio between these two activities. The correction for background, for cross over and for weight was carried out using Program B. This program was written to correct each value automatically and to express each value as a fraction of the value for the first specimen. In addition the fractional standard error of every value was calculated. A description, a program scheme, the mathematical basis, instructions for use, an annotated program and an example are included as Program B at the end of this section (method 22).

15. Calculation of rate of loss of albumin and activity at time of injection

The ratio between the activity due to albumin and the activity due to haemoglobin changed during the period following injection as radioactive albumin was lost from the circulation; initially there was no significant return of radio-active albumin either from synthesis or from the thoracic duct. The loss was therefore expected to follow an exponential decay in the initial period and it was found that an exponential regression fitted the observations satisfactorily during the period 10 - 45 minutes following injection. Calculation of the rate of loss and of the initial value (calculated value for time of injection) was carried out using Program C. This program was developed to allow the set of values and the corresponding regression equation to be represented graphically with the initial value normalised to 1.0. This allows visual comparison of a pre-operative albumin loss with an intraoperative albumin loss. The same program was used to calculate intercept values (y at x = 0) for blood volume calculations using the values for albumin counts and the values for haemoglobin counts. A description of the mathematical basis, instructions for use, an annotated program and an example are included as Program C at the end of this section (method 23).

16. Displaying the results

The results have been displayed graphically where applicable. The Degree of Extravasation of Albumin Relative To Haemoglobin (D.E.A.R.T.H.) is plotted as values for albumin, haemoglobin and ratio relative to an initial value for each measurement. Relative Albumin Transcapillary Escape rate (R.A.T.E.) is displayed as a slope starting at an initial value of 1.0.

17. Accuracy of the results

Errors arise from two sources. The standard error due to counting affects any radioactive estimation. This error is minimised by obtaining large counts. Additional errors may arise due to weighing errors, isotope decay, screening due to the container (test tube), positional variation in the counter and changes in the sensitivity of the counter due, for example, to voltage fluctuation. An estimate of how such errors might affect the ratio was made by counting ten samples of a thoroughly mixed stock solution containing two isotopes. The relative ratios calculated for these specimens (using Program B) are shown in the table on the following page.

Table: Accuracy check using prepared solution

Sample	Weight g.	^{125}I Channel 1	^{51}Cr Channel 2	^{125}I : ^{51}Cr Relative ratio from Program B
Background		1510	7249	
Standard		28321	691050	
1	1.967	383430	361649	1.000000
2	1.970	381113	362249	1.008210
3	1.964	380974	356767	0.992624
4	1.971	387157	359871	0.984673
5	1.964	379122	359599	1.006075
6	1.952	377514	358930	1.008662
7	1.981	386432	362402	0.993907
8	1.985	379705	364193	1.017867
9	1.943	380659	357529	0.995721
10	1.964	384279	361331	0.996733
			Mean Relative Ratio:	1.000447
			Variance:	0.000094
			Standard deviation:	0.00973
Calculated counting error (fractional standard error):				0.00240

17. Accuracy of the Results (cont)

The mean of the relative ratios is almost exactly 1.0 and their standard deviation is 0.00973. As the fractional standard error expected from the count in this experiment was only 0.00240, the contribution from other sources to the standard deviation = $\sqrt{(0.00973^2 - 0.00240^2)} = 0.0094$. (or 0.94%). This error has been taken into consideration with the error calculated for the count when preparing the graphs. Thus the 95% confidence band on each D.E.A.R.T.H. graph incorporates the calculated fractional standard error for the count and the standard deviation of 0.0094 expected from other sources. The 95% confidence bars preceding every graph represent only the counting errors. The numerical values for these errors are shown in the table on the following page.

In experiment 7, duplicate specimens were obtained throughout the experiment with four specimens initially and three for the next and for the final values. The discrepancies between replicate ratios were:

0.0026, 0.0073, 0.0094, 0.0047, 0.0068,

0.0021, 0.0201, 0.0011, 0.0212, 0.0043,

0.0062, 0.0015, 0.0158, 0.0120, 0.0084,

0.0031, 0.0012, and 0.0025. The mean discrepancy was 0.00725, and the maximum was 0.0212. This accords with the calculated 95% confidence limit for this experiment of +0.0198. In experiment 17 duplicate specimens were also obtained throughout the experiment. The discrepancies between duplicate ratios were:

0.0074, 0.0022, 0.0162, 0.0046, 0.0042,

0.0256, 0.0135, 0.0072, 0.0116, 0.0055,

0.0099, 0.0054. The mean discrepancy was 0.0094 and the maximum was 0.0256. This also accords reasonably with the calculated 95% confidence limit for the experiment of ± 0.0201.

Table: 95% Confidence limits for ratios in results

Case No.	95% Confidence limit of counting errors (Frac. Stand. Error x 2)	95% Confidence limit. calculated to include experimental error
2	±0.0125	±0.0226
3	0.0045	0.0194
5	0.0064	0.0199
6	0.0065	0.0199
7	0.0060	0.0198
9	0.0074	0.0203
10	0.0096	0.0212
12	0.0191	0.0268
13 control	0.0053	0.0196
13 expt	0.0055	0.0196
14	0.0062	0.0199
15	0.0103	0.0215
16	0.0063	0.0199
17	0.0069	0.0201
18 control	0.0047	0.0194
18 expt	0.0051	0.0195
21	0.0049	0.0195
22	0.0165	0.0250
23	0.0072	0.0202

18. The influence of isotope decay

For the isotopes employed in these experiments the following table shows the half life, the fractional decay rate and the error between consecutive samples when they are counted for 1000 seconds and for 500 seconds for each sample.

Isotope	Half life in days	Fractional rate of change per hour	Error Between Consecutive samples 100 sec count	500 sec count
^{131}I	8.05	-0.00359	0.100%	0.050%
^{51}Cr	27.8	-0.00104	0.0289%	0.0144%
^{125}I	60.0	-0.000482	0.0134%	0.0067%

When ^{131}I was used for the measurement of the relative albumin transcapillary escape rate, the shorter counting time employed and the simultaneous decay of ^{51}Cr reduce the decay error between samples to under 0.04%. Thus, if six samples were taken in 30 minutes, the error in the calculated escape rate per hour would be under 0.5%. As this error is small compared with both the rate being measured and the other sources of error, it has been ignored.

In other experiments the use of $^{125}1$ (whether with ^{51}Cr or without) reduced the error between samples due to decay to less than .010%, and even when in Method 1 25 samples were counted (about the largest number) the error between the first and last sample is less than 0.5%. Again this is small compared with the changes found and has therefore been ignored.

19. The influence of impurities

Used within the manufacturer's time limit, the quantity of unbound iodine in R.I.H.S.A. is stated to be less than 0.5% and the graphs obtained are consistent with there being insignificant quantities of unbound radio-active iodine. However, to confirm this on one occasion, the radiochemical purity of a sample of R.I.H.S.A. was tested. A sample of ^{131}I - R.I.H.S.A. was passed through a 30 cm. column of G-25 Sephadex (grade : fine, cross section area of column : 1 cm^2) using normal saline *as* an eluent. Fifty 1 ml. samples were collected and counted, and their activities plotted to provide a chromatogram. The areas corresponding to the R.I.H.S.A., to degradation products and to free iodide were compared. Less than 0.1% of activity was associated with the degradation products and free iodide combined.

In R.A.T.E. measurements, errors of approximately 0.1 might arise *in* the estimation of the percentage rate of loss per hour. This is a negligible effect and there is anyway no evidence of an initial, more rapid loss in the regression lines.

In D.E.A.R.T.H. measurements there is considerable time for the elimination of any unbound iodine and chromium. To confirm this in experiment 10, cells and plasma were separated and counted separately from samples taken before and after surgery. There was no measurable albumin in the washed red cells, and the 0.5% chromium measured in the plasma was smaller than the overall experimental error.

20. Discussion of the method

The methods described were designed to provide accurate information about the quantity of albumin remaining in the blood and about the rate at which albumin extravasation occurred.

The accuracy was intended to stem: (1) from calculating all values with respect to haemoglobin so that there would be automatic compensation for the effects of haemorrhage, internal fluid redistribution and infusion; (2) from counting both isotopes simultaneously in the mixture so that errors due to dilution, weighing and variation in counting time would be eliminated and so that errors due to decay and errors due to variation in shape and size of container would tend to be minimised.

Experimental determination of the overall error shows that when large counts are obtained (e.g. about a million) the standard deviation of the relative ratio is about 1.0, with smaller counts (e.g. 200,000) the larger counting error would raise the overall standard deviation to about 1.3. This has proved a satisfactory degree of accuracy to show the changes in the relative ratio.

The absence of effect on the relative ratio caused by contamination of the sample with infusion fluids is demonstrated in patients 3, 5, 6, 12 and 15 (see Results section). The samples taken at 1306 Case 3, at 1453 Case 5, at 1255 Case 6, at 1405 Case 6, at 1603 Case 12 and at 1100 Case 16 were withdrawn without previously discarding the infusion fluid in the dead space of the catheter. In no case was there any evidence that such errors in technique affect the relative ratio. That there was no effect on the relative ratio caused by rapidly infusing large volumes of intravenous fluid is similarly demonstrated in patients 7, 9, 10 and 18 (see Results section). The

samples taken at 1547, 1611 and 1613 Case 7, at 1210, 1228 and 1230 Case 9, at 1545 1600 and 1610 Case 10 and at 1100 Case 18 were withdrawn after rapid infusion. The marked fall in activity due to albumin is matched by the fall in activity due to haemoglobin and the relative ratio shows no marked charge.

The tendency for errors to cancel out is also demonstrated in patient 14 (see Results section). The series of samples taken at 1455, 1505, 1520, 1533 and 1555 show fluctuation which affects both isotopes without any similar irregularity in the ratio.

The principal disadvantages of the method are the time that elapses between taking the samples and being able to compute the results, and the length of time required to process the data. However the method is sensitive and, with it, it has been possible to measure the changes in the circulating albumin level (D.E.A.R.T.H.) and the rate at which extravasation is occurring (R.A.T.E.) even during surgery.

21a.

Description of Program A. Paper tape reading program written for the Olivetti 602 for use with the punched paper tape from the Wallac 2 channel auto gamma counter.

The number of cycles of counting and the largest specimen number are entered first. As the tape is read, each specimen is printed followed by the count in the channel being read. The count is added to the store indicated by the specimen number. After reading the preset number of cycles, the program prints in turn each specimen number followed by the sum of the counts from that specimen.

21 b. Instructions for Program A. Paper tape reading program

1. Load paper in reader
2. Enter the program into the Olivetti 602
3. Set both decimal wheels at zero
4. Press V to sum the counts from Channel 1
5. Enter number of cycles of counting (number of times each specimen has been counted)
6. Press S
7. Enter largest specimen number
8. Press S. Tape is read and values are summed and printed (see below)
9. Reload paper tape
10. Press Z to sum the counts from Channel 2
11. Enter number of cycles
12. Press S
13. Enter largest specimen number
14. Press S

Machine prints:

Specimen number followed by count for each specimen.

After last specimen has been read machine prints each specimen number followed by its total count.

Limitations:

Maximum number of specimens is 32 - determined by number of stores available. Maximum count accepted is 9,999,999 determined by capacity of stores.

21c.

Program A.

Paper Tape Reading Program.

	<
Press V for channel 1	V
	r ◊
Turn on sense switch "M"	/ •
G to /W	W
Press Z for channel 2	/Z
Turn off sense switch "M"	/ -
	/W
Clear numbered stores	r *
	a *
Enter number of cycles to count	S
	A S
Store number of cycles in Ab ...	b ↑
Enter largest specimen number ..	S
	A S
Store largest number in AB	B ↑
(and in B)	B ↑
	/Y
	A S
	B ↓
	B -
	A S
If AB ≠ B, go to aV	A V
	r ◊
	A S
	b ↓
	A ↑
	d ↓
No. of cycles to count -1	-
	A S
Store in Ab	b ↕
If all cycles have been recorded	R S
(Ab was = 0) go to aW	A W
	a V
	<
Read tape	B -
Print specimen number	◊
Store specimen number in B	B ↑
	<
Read tape	B ◊

	<
Read tape	B ◊
	<
Read tape	B X
If sense switch "M" is on, jump	/ S
to aY	A Y
	<
Read tape	B ↓
	<
Read tape	B X
	a Y
Print count	◊
	↓
	/ S
	B •
Add count to store indicated by	/ S
specimen number	B ↕
If sense switch "M" is on, jump	/ S
to aZ	A Z
	<
Read tape	B S
G to /Y	Y
	a Z
	<
Read tape	B ↕
Go to /Y	Y
	a W
Clear store B for counting	B *
	b V
	r ◊
Print specimen number (B)	B ◊
	/ S
Print store indicated by no. in B	B ◊
	A ↑
	d ↓
	↓
	B •
Increase count +1 in B	B ↕
	A S
	B -
	A ↓
If count has not reached largest	A S
specimen no., go to bV	B V
If sense switch "M" is on,	/ S
go to /Z ...	Z
If not, go to start	V

zz

22a.

Description of Program B written for the Olivetti 602 to analyse the activity in a two isotope mixture

The program corrects every value for background activity and calculates the activity per gram and cross over ratio for the isotope standards. For every specimen the program calculates both isotopes' activity per gram and the ratio between these two activities. For the first specimen the results are stored as reference values; for subsequent specimens results are compared with and expressed as fractions of the values for the first specimen (relative activities and relative ratios).

22b. Scheme of Program B. Two-isotope analysis program.

Scheme of computer program

1. Background activity for each channel stored in D E,

2. Data input subroutine:

   Counts corrected for background in B C

   Their fractional standard errors squared in ..... b c

3. Channel 1 standard (p). Go to data input subroutine

   Cross over ratio Xp in..... d

   Its fractional standard error squared $(fXp)^2$ in AB

   If wt. of standard entered, print activity (counts per gram).

4. Channel 2 standard (q). Go to data input subroutine

   Cross over ratio Xq in e

   Its fractional standard error squared $(fXq)^2$ in AC

   If wt. of standard entered, print activity (counts per gram).

5. Calculate (1 - product of the cross over ratios)

   i.e. (1 - Xp.Xq) and store in . AD

   Its fractional standard error in ..... AE

6. Each specimen. Go to data input subroutine.

   Counts corrected for background and cross over in B C

   Their fractional standard errors in ..... b c

   If wt. of specimen entered: counts / wt in ..... B C

7. For specimen 1 only store B, C, in ..... AF Ae

   Calculate ratio B/C or C/B and store in Ad

8. For all specimens

   Print counts (per gram) for each isotope and ratio between them, Also print relative activity and relative ratio (compared with specimen 1).
   Print the fractional standard error of each value.

So that the values may be compared with Program B, the stores used are given in brackets thus: (AC)

Where P, Q are the respective counts in Channel 1 and Channel 2 — Stores used

P_b Q_b are the counts from the background (D, E)

P_p Q_p are the counts from the Channel 1 standard (p)

P_q Q_q are the counts from the Channel 2 standard (q)

P_r Q_r are the counts from the specimen

P_s Q_s are the counts from the specimen corrected for background (B, C)

P_t Q_t are the counts due to each isotope (corrected for background and crossover)

X_p is the cross over ratio for standard p (Channel 2/ Channel 1) (d)

X_q is the cross over ratio for standard q (Channel 1/ Channel 2) (e)

f stands for the fractional standard error, thus:

$f(X_p)$ stands for the fractional standard error of X_p

Cross over ratios, and their fractional standard errors

$$X_p = (Q_p - Q_b) / (P_p - P_b) \quad (d)$$

$$(fX_p)^2 = (P_p + P_b) / (P_p - P_b)^2 + (Q_p + Q_b) / (Q_p - Q_b)^2 \quad (AB)$$

$$X_q = (P_q - P_b) / (Q_q - Q_b) \quad (e)$$

$$(fX_q)^2 = (P_q + P_b) / (P_q - P_b)^2 + (Q_q + Q_b) / (Q_q - Q_b)^2 \quad (AC)$$

$(1 - X_p.X_q)$ Calculated and stored (AD)

$$\left(f(1 - X_p.X_q)\right)^2 = \left(\sqrt{(fX_p)^2 + (fX_q)^2}.X_p.X_q / (1 - X_p.X_q)\right)^2 \quad (AE)$$

$$P_t = (P_s - X_q.Q_s) / (1 - X_p.X_q) \quad \text{(see method section 12)} \quad (B)$$

$$fP_t = \sqrt{\left(\sqrt{P_r+P_b+X_q.Q_s+(X_q.Q_s)^2((fX_q)^2+(fQ_s)^2)/(P_s-X_qQ_s)}\right)^2+(f(1-X_p.X_q))^2} \quad (b)$$

$$Q_t = (Q_s - X_p.P_s) / (1 - X_p.X_q) \quad \text{(see method section 12)} \quad (C)$$

$$fQt = \sqrt{\left(\sqrt{Q_r+Q_b+X_p.P_s+(X_p.P_s)^2((fX_p)^2+(fP_s)^2)/(Q_s-X_p.P_s)}\right)^2+ (f(1-X_p.X_q))^2} \quad (c)$$

Notes:

The fractional standard error, e.g. for fP_t, was derived by summing the variance of $P_s(=P_r + P_b)$, of $X_q.Q_s$ and of the estimate of $X_q.Q_s$. From this was calculated the square of the fractional standard error. This was added to the square of the fractional standard error of $(1 - X_pX_q)$. The square root of the result was the fractional standard error of the corrected count.

22d, Instructions for Program B. Two-isotope analysis program

1. Enter program into Olivetti 602.
2. Set upper decimal wheel at zero, lower at 6.
3. Press V.
4. Enter experiment number, press S.
5. Enter Channel 1 background count, press S.
6. Enter Channel 2 background count, press S.
7. Enter identification of first isotope standard, press S.
8. Enter Channel 1 count for first isotope standard, press S.
9. Enter Channel 2 count for first isotope standard, press S.
10. Machine prints: cross over ratio fractional standard error
11. Enter weight of first isotope standard if weighed, and/or press S.
12. Machine prints: counts (per gram) of standard.
13. For second isotope standard: repeat steps 7 – 12.
14. Enter specimen number, press S.
15. Enter Channel 1 count for specimen, press S.
16. Enter Channel 2 count for specimen, press S.
17. Enter weight of specimen, press S.
18. Press Y to calculate ratio as Channel 1/Channe1 2, or press Z to calculate ratio as Channel 2/Chanel 1.
19. Machine prints: counts per gram for Channel 1 relative activity (fraction of the 1st specimen) the fractional standard error of these values.

    counts per gram for Channel 2 relative activity (fraction of the 1st specimen) the fractional standard error of these values.

    the ratio between channels as selected in step 16 relative ratio (fraction of the 1st specimen) the fractional standard error of these ratios.
20. For remaining specimens: repeat steps 14 – 17.

Limitation: Ratio between Channel 1 and Channel 2 for 1st specimen must be less than 10 to be accommodated by storage register. If exceeded it is then necessary to select the reciprocal ratio (i.e. press Z instead of Y, or vice versa).

Note 1: A count exactly equal to the background causes division by zero in the calculation of the standard error. Increase such a count by 1.

Note 2: Where no ^{125}I standard was counted, enter dummy values, e.g. for step 7 enter 0, for step 8 enter a large activity such as 1,000,000 and for step 9 a minimal activity such as background 1 (see note 1).

22e.
Program B
Two-Isotope Analysis Program

Press V to start	(V)
	r ◊
	a -
Sense switches "A", "M", off ..	/ -
Enter experiment no.	S
	r ◊
Enter Channel 1 background	S
In D	D ↑
Enter Channel 2 background	S
In E	E ↑
1st standard. Go to input sub.	C ∫
	C ↓
Channel 2/Channel 1	B ÷
Stand. 1 cross-over ratio in d	d ↕
Print ratio	d ◊
	b ↓
$\Sigma(f.s.e.)^2$	c +
Go to second subroutine	D ∫
	A S
$\Sigma(f.s.e.)^2$ in AB	B ↑
	↓
$\sqrt{AB}$	A ∫
Print f.s.e. of ratio	A ◊
	r ◊
	*
Enter wt. of 1 or press S	S
	↓
	R S
If no wt., jump to aV	A V
	B ↓
Channel 1/wt.	÷
Print counts per gram	A ◊
	a V
2nd standard. Go to input sub.	C ∫
	B ↓
Channel 1/Channel 2	C ÷
Stand. 2 cross-over ratio in e	e ↕
Print ratio	e ◊
	b ↓
$\Sigma(f.s.e.)^2$	c +
Go to second subroutine	D ∫
	A S
$\Sigma(f.s.e.)^2$ in AC	C ↑
	↓
$\sqrt{AC}$	A ∫
Print f.s.e. of ratio	A ◊
	r ◊
	*
Enter wt. of 2 or press S	S
	↓
	R S
If no wt., jump to aW	A W
	C ↓
Channel 2/wt.	÷
Print counts per gram	A ◊
	a W
Cross-over ratio 1(d)	d ↓
Times cross-over ratio 2(e)	e X
	A ↑
	d ↓
	↕
	-
	A S
(1 - d.e) in AD	D ↕
	A S
$\Sigma(f.s.e.)^2$ of ratio 1 (AB)	B ↓
	A S
$+\Sigma(f.s.e.)^2$ of ratio 2 (AC) ...	C +
$\sqrt{AB + AC}$	A ∫
	d X
d.e.$\sqrt{AB + AC}$	e X
	A S
÷ (1 - d.e)	D ÷
Squared	A X
	A S
= $(f.s.e.)^2$ of (1 - d.e)	E ↕
	b V
Specimen. Go to input sub.	C ∫
	C ↓
	e X
	r ↕
Channel 2 times cross over in F	F ↑
	B ↓
	F -
	r ↕
(Channel 1 - F) in f	f ↑

Program B (cont.)

$(f.s.e.)^2$ Channel 2	c ↓
	A S
+ $(f.s.e.)^2$ of ratio 2	C +
	F X
Times F^2	F X
+ F	F +
	B +
+ Count Channel 1	D +
+ Background Channel 1	+
Square root	A √
÷ (Channel 1 - F)	f ÷
Squared	A X
	A S
+ $(f.s.e.)^2$ of (1 - d.e)	E +
Square root.....	A √
$(f.s.e.)^2$ of Channel 1 in b	b ↑
$(f.s.e.)^2$ Channel 1 in f	f ↑
	A S
(Channel 1 - F) ÷(1 - d.e.) ...	D ÷
	r ↑
	B ↓
Corrected Channel 1 in B	B ↑
	d X
	r ↑
Channel 1 times cross over in F	F ↑
	C ↓
	F -
	r ↑
	f ↓
Channel 2 - F in f	f ↑
$(f.s.e.)^2$ Channel 1	A S
+ $(f.s.e.)^2$ of ratio 1	B +
	F X
Times F^2	F X
+ F	F +
	C +
+ Count (Channel 2)	E +
+ Background (Channel 2)	+
Square root	A √
÷ (Channel 2 - F)	f ÷
Squared	A X
	A S
+ $(f.s.e.)^2$ of (1 - d.e.)	E +
Square root	A √
(f.s.e.) of Channel 2 in c	c ↑
	f ↓
	A S
(Channel 2 - F) ÷ (1 - d.e.) ...	D ÷
	r ↑
Corrected Channel 2 in C	C ↑
	*
Enter wt. of specimen or press S	S
	↓
	R S
If no wt., jump to aY	A Y
	B ↑
Channel 1 ÷ wt.	÷
	r ↑
	B ↓
Channel 1 counts/gram in B	B ↑
	C ↑
Channel 2 ÷ wt.	C ÷
	r ↑
Channel 2 Counts/gram in C	C ↑
	a Y
If sense switch "M" is on, jump	/ S
to aZ ...	A Z
	S
Press Y for: Ratio = Ch 1/ Ch 2	/ Y
Turn on sense switch "M"	/ +
Turn off sense switch "A"	a -
	B ↓
	C ÷
	A S
For 1st specimen: Ch 1/Ch 2 in Ad	d ↑
Jump to bZ	B Z
Press Z for: Ratio = Ch 2/Ch 1	/ Z
Turn on sense switch "M"	/ +
Turn on sense switch "A"	a +
	C ↓
	B ÷
	A S
For 1st specimen: Ch 2/Ch 1 in Ad	d ↑

Program B final section

	b Z
	B ↓
	A S
Specimen 1 Channel 1 in AF	F ↕
	C ↓
	A S
Specimen 1 Channel 2 in Ae	e ↕
	a Z
	B ↓
	A S
Channel 1 ÷ AF	F ÷
Print Channel 1	B ◊
Print Channel 1 ÷ AF	A ◊
Print (f.s.e.) of Channel 1	b ◊
	r ◊
	C ↓
	A S
Channel 2 ÷ Ae	e ÷
Print Channel 2	C ◊
Print Channel 2 ÷ AF	A ◊
Print (f.s.e.) of Channel 2	c ◊
	r ◊
If sense switch "A" is on, jump	a S
to bY .	B Y
	B ↓
Channel 1/Channel 2	C ÷
Jump to bW	B W
	b Y
	C ↓
Channel 2/Channel 1	B ÷
	b W
Print ratio	A ◊
	A S
	d ÷
Print ratio ÷ Ad	A ◊
	b ↓
	A X
Channel 1 $(f.s.e.)^2$ in c	c ↕
	A X
+ Channel 2$(f.s.e)^2$	c +
Square root	A √
Print (f.s.e.) of ratio	A ◊
	B V

Input Subroutine	c √
	r ◊
	r ◊
Enter specimen time (or no.) ...	S
	r ◊
Enter Channel 1 count	S
	B ↑
	B ↓
	D -
	r ↕
	B ↓
Chan 1: Count - background in B	B ↑
	D +
	B ÷
Chan 1 count + background/B^2 ...	B ÷
Go to second subroutine	D √
$(f.s.e.)^2$ of B in b	b ↑
Enter Channel 2 count	S
	C ↑
	C ↓
	E -
	r ↕
	C ↓
Chan 2: Count - background in C	C ↑
	E +
	C ÷
Chan 2 count + background/C^2 ...	C ÷
Go to second subroutine	D √
$(f.s.e.)^2$ of C in c	c ↑
	r ◊
End input subroutine	c √
Second subroutine	d √
	A ↑
	D S
	r ↓
	↕
10 - number	-
	A S
If number < 10, go to cZ	C Z
	↕
Integer value	r ↕
	c Z
End second subroutine	d √
	/ V

22f. Program B example

```
Press V ..........................  V

Enter Expt. No.                23    S

Enter Ch 1 background        1399    S
Enter Ch 2 background        2902    S

125I
Enter No. of 1st Stnd.          1    S

Enter Ch 1 count           147310    S
Enter Ch 2 count             3001    S

Prints ratio             0.000678    d◊
Prints f.s.e.            0.776074    A◊

Press S ..........................  S

51Cr
Enter No. of 2nd Stnd.          3    S

Enter Ch 1 count             9889    S
Enter Ch 2 count           154678    S

Prints ratio             0.055937    e◊
Prints f.s.e.            0.012727    A◊

                                     S

Specimen 1
Enter time                    805    S

Enter Ch 1 count            41680    S
Enter Ch 2 count           103147    S

Enter wt.                   2.097    S
Press Y  (Ch 1 ÷ Ch 2)               Y
Cts/g Ch 1       16535.000000    B◊
Relative to spec 1       1.000000    A◊
f.s.e.                   0.006633    b◊

Cts/g Ch 2       47792.000000    C◊
Relative to spec 1       1.000000    A◊
f.s.e.                   0.003162    c◊

Ratio                    0.345978    A◊
Relative to spec 1       1.000000    A◊
f.s.e.                   0.007211    A◊

Specimen 2
Enter time                    825    S

Enter Ch 1 count            40166    S
Enter Ch 2 count            98761    S

Enter wt.                   2.083    S
                 16037.000000    B◊
(Results                 0.969882    A◊
 as for                  0.006782    b◊
 spec. 1)
                 46008.000000    C◊
                          0.962671    A◊
                          0.003316    c◊

                          0.348569    A◊
                          1.007488    A◊
                          0.007416    A◊

Specimen 3
As for spec. 2                905    S

                            40680    S
                           102620    S

                             2.074    S
                 16251.000000    B◊
                          0.982824    A◊
                          0.006782    b◊

                 48068.000000    C◊
                          1.005775    A◊
                          0.003162    c◊

                          0.338083    A◊
                          0.977180    A◊
                          0.007348    A◊
```

23a. Description of Program C written for the Olivetti 602 to calculate the regression log y (activity): x (time).

For a series of pairs y, x; the program calculates the regression coefficients for the equation $\log_e y = \log_e A - B.x$, i.e. $y = e^{(\log_e A - B.x)}$. The program prints the number of points, the degrees of freedom, the natural log of the intercept (log y at x = 0), the intercept (y at x = 0), the slope (B) which represents the fractional rate of change per unit time, the correlation coefficient, and the half life. Each value of x is then reprinted with the y value and the regressed y value both to the normalised scale (y = 1 when x = 0) followed by the y value and the regressed y value both to the original scale.

23b. Mathematical basis for Program C : Calculation of regression equation

To calculate regression equation $y = e^{(\log_e A - B.x)}$

Where y = counts due to isotope or ratio between albumin and haemoglobin

X = time in minutes after injection at x = 0

A = y intercept (calculated activity for x = 0)

B = slope (fractional escape rate of isotope per minute)

For each pair of values (y, x) the following values are calculated and added to a progressive total for each function:

Function:

$\log_e y$	RE
$(\log_e y)^2$	Re
$x.\log_e y$	Rd
X	RF
2	
X^2	Rf
N (Number of pairs)	RD

Then:

$X = \sum(X^2) - (\sum X)^2/N$	Rf
$Y = \sum(\log_e y)^2 - (\sum \log_e y)^2/N$	Re
$Z = \sum(x.\log_e y) - (\sum x.\sum \log_e y)/N$	Rd
mean value of $x = \sum X/N$	RF
mean value of $\log_8 y = \sum(\log_E y)/N$	RE

And:

$\log_e A$ = mean($\log_e y$) − mean(x) . Z/X

B = Z/X

r = z/√(X.Y) (correlation coefficient)

Half life = ($\log_e 0.5$)/B

Degrees of freedom = N - 2.

23c. Instructions for Program C : Calculation of regression equation

1. Enter the program card side 1 into the Olivetti 602
2. Set upper decimal wheel at zero, lower at 6
3. Press V to enter data pairs (y, x)
4. Enter y, press S
5. Enter x, press S
6. Repeat steps 4 and 5 for each pair of values
7. Press Z after last pair of values
8. Enter program card side 2
9. Press V to print results
10. Machine prints:

    Number of points

    Degrees of freedom

    Log of A (log of y intercept at x = 0)

    A (intercept at x = 0)

    B, slope (fractional rate of change per unit time)

    r, correlation coefficient

    Half life
11. Machine prints for each specimen:

    x, time

    y normalised (to the scale y = 1 when x = 0)

    y computed from the regression equation (normalised)

    Y

    y computed from the regression equation
12. Enter any x value
13. Machine prints:

    y computed from the regression equation (normalised)

    Y computed from the regression equation

Repeat steps 12, 13 as required.

23d.

Program C

Regression calculation log y:x

Program Card side 1

Press V to enter y,x, values ...	V
Clear numbered stores	a *
	r *
	a V
	r ◊
Enter y	S
	↓
	/ *
Natural logarithm of y	A Y
Log y in B	B ↕
	B ↓
	a ↕
Express in floating point	/ ↕
	r S
Log y in store "N"	D ↕
	R S
	E ↓
	B *
	R S
Σ (log y) in RE	E ↕
	↓
	X
	R S
	e *
	R S
$\Sigma(\log y)^2$ in Re	e ↕
	A ↑
	D S
	r ↓
10	↓
	R S
10 + N	D *
Enter x	S
	/ S
x in store (N + 10)	A ↑
	B ↓
x.log y	X
	↕
	R S
	d ↕
	*
	R S
Σ (x.log y) in Rd	d ↕
	A X
	R S
	F ↕
	*
	R S
$\Sigma(x)$ in RF	F ↕
	R S
	f *
	R S
$\Sigma(x^2)$ in Rf	f ↕
	A ↑
	d ↓
1	↓
	R S
	D *
	R S
(N + 1) in RD	D ↕
Loop to aV	A V
Press Z after last pair entered	/ Z
	R S
	F ↓
$(\Sigma x)^2$	A X
	R S
$(\Sigma x)^2/N$	D *
	A ↕
	R S
	f *
	R S
$X = \Sigma(x^2) - (\Sigma x)^2/N$ in Rf	f ↕
	R S
	E ↓
	A X
	R S
	D *
	A ↕
	R S
$Y = \Sigma(\log y)^2 - (\Sigma \log y)^2/N$ ..	e *
	R S
In Re	e ↕

Program C

Card side 1 cont:	R S
	E ↓
	R S
	F X
	R S
	D ↑
	A ↕
	R S
$Z = \Sigma(x.\log y) - (\Sigma x.\Sigma \log y)/N$	d ↑
	R S
In Rd	d ↕
	R S
	F ↓
	R S
	D ↑
	R S
$\bar{x}$ in RF	F ↕
	R S
	E ↓
	↑
	R S
$\overline{\log y}$ in RE	E ↕
	R S
	f ↓
	R S
	e X
$\sqrt{X.Y}$	A √
	↕
	R S
	d ↓
	↑
Correlation coefficient:	R S
(In Rd) $r = Z/\sqrt{X.Y}$	d ↕
	R S
	f ↑
	R S
Slope (log y/x) = Z/X in RF	F ↕
	R S
$\bar{x}.Z/X$	F X
	A ↕
	R S
$\overline{\log y}$	E ↑
	R S
Log intercept=$\overline{\log y}-\bar{x}.Z/X$ in RD	D ↕
N (no. of points) in A	/V
(End of side 1)	

Program C. Card side 2

Regression calculation log y:x

Press V to finish calculation	V
Print N (no. of points)	A ◊
	A ↕
2	d ↕
	-
Print degrees of freedom	R ◊
	A *
	R S
	D ↑
Print natural log intercept ...	◊
	r ◊
	/ *
Exponent	A Z
	a X
Print intercept (y at x = 0) ..	A ◊
	R S
Store intercept in Rf	f ↕
	R S
Slope	F ↓
	B ↕
Print slope (log y/x)	B ◊
	A *
	R S
	d ↑
Print correlation coefficient	R ◊
	r ◊
	A ↕
	B -
	B ↓
	B ↕
	B *
	B X
(Natural log 0.5) -0.69315 ..	f S
	↓
	R S
÷ slope	F ↑
	b ↕
Print half-life	b ◊

Program C
Card side 2 cont:

	R S
Clear store RE for counting ...	E *
	/W
	r ◊
	A ↑
	D S
10	r ↓
	↓
	R S
(N + 10)	E +
	/ S
	A ↓
Print x (from store (N + 10))	A ◊
	R S
Store x in Re	e ↕
	r S
Log y (from store N)	E ↓
	r ↕
From floating to fixed point ..	a X
	R S
- Log intercept	D -
	/ *
Exponent	A Z
	a X
Print y normalised	A ◊
	R S
Store in Re	e ↕
	R S
x.slope	F X
	/ *
Exponent	A Z
	a X
	↕
Print computed y normalised ...	◊
	↕
	R S
Times intercept	f X
	R S
Computed y in Re	e ↕
y normalised times intercept ..	X
Print y	A ◊
	R S
	e +
Print computed y	◊

	A ↑
1	d ↓
	↓
	R S
	E +
	R S
(N + 1) in RE	E ↕
	r S
	E ↓
	A ↓
Test: Is store (N + 1) filled?	A S
If so; return to /W	W
Press Y to compute y from x ...	/Y
	r ◊
Enter x	S
	↓
	R S
x.slope	F X
	/ *
Exponent	A Z
	a X
	↕
Print computed y normalised ...	◊
	↕
	R S
Times intercept	f X
	↕
Print computed y	◊
Return to /Y	Y
	/V

23e.

Program C example

Calculation of Regression equation

Patient 20, during surgery.

Insert Program card side 1

Press V		V
Enter ratio (y)	0.539784	S
Enter time (x)	5	S
"	0.517573	S
	10	S
"	0.537990	S
	15	S
"	0.495036	S
	25	S
"	0.486764	S
	35	S
"	0.468195	S
	46	S

After final values press Z .... Z

Insert Program card side 2

Press V to print:		V
No. of points	6.000000	A ◊
Degree of freedom	4	R ◊
Log y intercept	-0.601607	◊
y intercept	0.547936	A ◊
slope	-0.003437	B ◊
Correl. coefft.	-0.941463	R ◊
Half-life	201.672970	b ◊
For every value:		
Time	5.000000	A ◊
y value normalised	0.985149	A ◊
y computed "	0.982965	◊
y value	0.539798	A ◊
y computed	0.538601	◊
"	10.000000	A ◊
	0.944605	A ◊
	0.966235	◊
	0.517583	A ◊
	0.529434	◊
"	15.000000	A ◊
	0.981878	A ◊
	0.949761	◊
	0.538006	A ◊
	0.520408	◊
"	25.000000	A ◊
	0.903478	A ◊
	0.917685	◊
	0.495048	A ◊
	0.502832	◊
"	35.000000	A ◊
	0.888387	A ◊
	0.886672	◊
	0.486779	A ◊
	0.485839	◊
"	46.000000	A ◊
	0.854495	A ◊
	0.853763	◊
	0.468208	A ◊
	0.467807	◊
Frac. change/min.	0.003437	↓
x 6000	6000	X
% change/hour	20.622000	A ◊

RESULTS

1. Introduction

The radio-active counts from each experiment were printed automatically on the teleprinter attached to the auto gamma counter. As all the specimens were counted repeatedly, a mass of data was obtained (see example in back pocket). The punched paper tape which the teleprinter also produced was subsequently processed using program A (Method section 21), and this automatically produced summated counts for every specimen. These total counts, the times at which the specimens were taken and the weight of blood in each specimen are recorded in the tables of results (see Results section 2).

The counts recorded in the tables were processed with program B (Method sections 8, 14_2 17 and 22) to obtain activity per gram, relative activity, relative ratio and counting error. These values were used to construct the D.E.A.R.T.H. graphs (see Results section 3). These graphs show the alteration in albumin activity, haemoglobin activity and relative ratio (albumin/haemoglobin) each normalised to an initial value of 1.0.

The R.A.T.E. graphs (Results section 4) were derived using program C (Method sections 9, 15 and 23) to calculate the regression: log of relative ratio against time for the period following the injection of radio-active tracer.

The plasma and blood volume calculations (Results section 5) were based on values obtained from Results section 2 and 3. The activity per *gram* of patients' blood at the time of injection (t=0) was calculated from the activities during the period following injection by using the regression program C (Method sections 15 and 23).

In two cases in which there was marked haemodilution (see (D.E.A.R.T.H. graphs for patients 7 and 10), haematocrits were obtained for every specimen of blood counted; the radioactivity appeared to fall more rapidly than the haematocrit. The relationship between this apparent change in red cell activity and the albumin concentration was tested using a linear regression equation (Results section 6). In sections 7, 8, 9,10 the results obtained are reviewed and summarised.

2. Tables of Results

The tables on the following pages show:

Times at which specimens were taken,

Weights of specimens,

Activity (uncorrected) in each counting channel.

RESULT OF EXPERIMENT NUMBER 2

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Channel 1 (^{51}CR)	Channel 2 (^{125}I)
Background	6665	3899
^{51}Cr Stnd.	9055490	450835
1425	792265	103999
1430	796397	103634
1435	793773	104035
1438	795151	103315
1442	790709	102647
1452	800975	103023
1508	814096	104317
1523	815660	102475
1529	820668	103156
1543	834759	103625
1554	823155	102907

Note: in this table the values were typed in the wrong columns

RESULT OF EXPERIMENT NUMBER 3

TIMES WEIGHTS AND SUMMATED COUNTS

Time	~~Channel 1 (^{51}CR)~~	~~Channel 2 (^{125}I)~~	Channel 1 (^{51}CR)	Channel 2 (^{125}I)
Background	~~3306~~	~~6111~~	6111	3306
^{51}Cr Stnd	~~110920~~	~~2217902~~	2217902	110920
1253	~~333100~~	~~941257~~	941257	333100
1306	~~314750~~	~~897248~~	897248	314750
1329	~~330522~~	~~939090~~	939090	330522
1406	~~321212~~	~~918260~~	918260	321212
1420	~~330235~~	~~956910~~	956910	330235
1440	~~321356~~	~~932141~~	932141	321356
1506	~~317261~~	~~943098~~	943098	317261
1515	~~318671~~	~~948245~~	948245	318671
1533	~~306199~~	~~931766~~	931766	306199
1559	~~307565~~	~~943721~~	943721	307565
1618	~~301890~~	~~924171~~	924171	301890

RESULT OF EXPERIMENT NUMBER 5

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		7578	3540
^{51}Cr Stnd		1198814	57076
1418	2.0705	517104	187521
1418	2.0605	520396	187080
1418	2.0688	521232	188614
1453	2.0781	487893	177877
1537	2.0790	532373	186747
1537	2.0976	523562	189027
1556	2.0662	515169	185123
1609	2.0724	518356	185349
1635	2.0929	520601	186150
1658	2.0952	524399	184243
1805	2.1086	513513	183226
2115	2.0844	498548	177757
3420	2.0723	528851	176922

RESULT OF EXPERIMENT NUMBER 6

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		4904	2191
^{51}Cr Stnd		556876	25833
1255	2.0731	203080	179706
1300	2.0662	232815	203581
1405	2.0678	212115	185483
1415	2.0710	201891	182370
1416	2.0544	238753	206338
1417	1.8174	211789	180740
1418	2.0709	236587	210810
1425	2.0495	199267	173718
1426	2.0645	238492	205563
1430	2.0727	243367	203068
1431	2.0667	244507	203268
1453	2.0774	241178	202907
1508	2.0726	249216	199674
1515	2.0532	241785	197955
1530	2.0935	248626	197053
1545	2.0817	248537	193595

RESULT OF EXPERIMENT NUMBER 7

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		5961	3073
^{51}Cr Stnd		1163014	56786
1351	2.1012	298017	255055
1353	2.1038	292180	251598
1355	2.0823	292299	251205
1357	1.9107	276724	236112
1359	2.0718	297548	249379
1401	2.0812	294659	251667
1403	2.0480	289387	247371
1430	2.0506	276464	239197
1432	2.0265	271196	235535
1452	2.0533	278496	238302
1454	2.0502	279523	237796
1508	2.0646	275245	233936
1510	2.0215	270316	229396
1532	2.0264	271792	228707
1534	2.0259	267402	228324
1546	2.0360	244247	211902
1548	1.9854	239697	210129
1611	1.9846	227302	195608
1613	2.0503	219877	190620
1633	2.0251	257403	211533
1634	2.0298	254979	210151
1635	2.0278	255121	210551

RESULT OF EXPERIMENT NUMBER 9

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		5920	2458
^{51}Cr Stnd		153976	56786
0835	2.0851	217578	141809
0835	2.0955	198230	131654
0835	2.0907	218373	142614
0835	2.0341	213281	139050
0910	2.0733	224676	142903
0945	2.0776	214311	139575
1005	2.0771	217429	137422
1045	0.5366	61386	37448
1115	2.0837	226094	130458
1140	2.1454	230676	135190
1210	2.0510	196769	114186
1227	2.0443	200835	115063
1228	1.9593	193034	110192
1229	2.0801	203229	118971
1230	1.8652	182845	106713

RESULT OF EXPERIMENT NUMBER 10

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		3707	1453
^{51}Cr Stnd		145043	6954
1415	2.0868	112021	92213
1415	2.0835	111636	92214
1415	2.0640	110149	91172
1445	2.0589	106348	88833
1500	2.0290	100934	84391
1515	2.0233	101950	85387
1530	2.0430	103785	84606
1545	2.0312	101230	83503
1600	2.0434	88547	74246
1610	2.0337	85209	71884
1625	2.0694	90855	77423
1640	1.9475	85889	72247
1655	2.0691	91871	76676
Separated samples:			
1415, cells		94460	4843
1415, plasma		4301	87454
1655, cells		85339	4441
1655, plasma		4242	77279

RESULT OF EXPERIMENT NUMBER 12

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		1928	854
^{51}Cr Stnd		411592	14995
1421	1.876	48689	21038
1422	2.124	55140	23460
1423	2.020	52719	22992
1424	2.023	52435	22796
1550	1.975	51751	21625
1603	1.975	49066	20493
1619	1.965	52246	21649
1627	1.919	51473	21267
1658	1.978	51943	21895
1704	1.938	50292	21036
1714	1.841	49838	19963
1725	1.954	52405	20722

RESULT OF EXPERIMENT NUMBER 13

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		12666	2844
^{51}Cr Stnd		974603	41107
17th January 1974			
1345	2.083	396572	257861
1345	2.103	403077	258670
1345	2.075	396210	257416
1345	2.004	387519	246266
1400	2.058	389773	250132
1415	2.063	389137	249242
1430	2.006	374642	238039
1445	2.107	392055	252080
1500	2.084	388599	248319
18th January 1974			
1405	2.065	370661	221927
1405	2.082	374940	226470
1405	2.071	371972	226307
1405	2.061	371762	225577
1427	2.064	364146	220166
1445	2.055	356607	217705
1500	2.093	360283	219270
1515	2.070	353140	214302
1535	1.982	335489	203148
1555	2.039	343584	206576
1620	2.002	327456	197623
1625	2.039	335379	197230
1635	1.820	305518	177061
1652	2.065	346676	203645

RESULT OF EXPERIMENT NUMBER 14

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		12298	2724
^{51}Cr Stnd		491345	21268
1330	2.093	339383	194127
1347	2.100	338046	193482
1400	2.101	339664	194845
1440	2.061	353004	192571
1455	2.061	319711	175867
1505	2.088	333764	183715
1520	2.078	324590	178228
1533	2.060	339144	184453
1555	2.077	328499	175553
1610	2.046	336923	176194
1620	2.059	338732	175896
1635	2.057	336955	178688

RESULT OF EXPERIMENT NUMBER 15

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		8553	1758
^{51}Cr Stnd		244728	10638
1445	2.022	163054	71784
1500	2.007	161539	69895
1525	2.050	166861	70666
1545	2.051	168623	71307
1605	2.043	160503	69342
1620	2.097	165992	70035
1635	2.122	174150	72885
1655	2.112	171831	70662
1705	2.104	167608	69362
1720	2.111	169948	69922
1740	2.105	171475	69428
1750	2.090	169215	67499
1810	2.069	165965	67320

RESULT OF EXPERIMENT NUMBER 16

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		8553	1758
^{51}Cr Stnd		244728	10638
0840	2.030	365255	148404
0850	2.135	384979	156494
0945	2.102	382795	152595
1000	2.093	377222	151008
1017	2.070	374034	147309
1030	2.087	373005	144177
1045	2.087	375340	145810
1100	1.988	327473	124970
1115	2.057	374672	141737
1130	2.086	378074	143480

RESULT OF EXPERIMENT NUMBER 17

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		18000	2430
^{51}Cr Stnd		688249	28566
0828	2.141	93429	236688
0828	2.108	93014	233686
0850	2.079	93895	236090
0850	2.138	95303	239912
0910	2.049	91250	225528
0910	1.960	88025	219168
0935	2.087	90438	226248
0935	1.987	87752	216956
0955	2.118	92592	223873
0955	2.106	92410	222376
1018	2.054	90400	219346
1018	2.084	92660	220269
1045	2.015	89846	211780
1045	2.080	90593	216945
1055	2.055	90609	213832
1055	2.096	91226	217242
1110	2.087	91873	214719
1110	2.081	91452	216112
1150	2.092	92000	214574
1150	2.113	91986	215779
1225	2.015	87211	204355
1225	2.041	88993	207419
1240	1.996	88033	205689
1240	2.017	88371	205520

RESULT OF EXPERIMENT NUMBER 18

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		19237	2780
^{51}Cr Stnd		829141	35017
3rd April 1974			
0815	2.111	667548	321973
0830	2.063	661679	309715
0845	2.055	656258	307642
0900	2.058	659573	308172
0915	2.072	664852	308885
0930	2.068	673037	313908
0945	2.080	666228	310492
1000	2.014	641139	295919
4th April 1974			
0815	2.077	621469	275186
0830	1.994	598212	258508
0850	2.016	620341	265871
0915	2.086	624246	266184
0930	2.049	628137	262241
0945	2.075	622219	263083
1000	2.082	613041	260926
1015	2.050	621943	256658
1030	1.940	592180	240547
1045	1.938	581717	237511
1100	1.946	417698	166227
1115	1.935	468202	190313
1125	1.962	488793	195507
1145	2.032	520203	206930

RESULT OF EXPERIMENT NUMBER 19

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{131}I)	Channel 2 (^{125}I)	Haematocrit (Avge. Of 4)
Background		3149	3601	
^{131}I Stnd		105963	17798	
1341	1.988	3272	49828	37.i0
1345	1.824	3266	46878	36.87
1350	2.110	3225	51537	37.17
1355	2.084	3199	49699	37.05
1400	2.097	3062	50001	37.37
1405	2.052	3140	49551	37.00
1410	2.057	3257	49720	36.75
1544	2.008	108198	48716	37.37
1548	1.926	126343	49849	37.37
1553	2.006	128398	50425	37·75
1558	2.090	130648	51850	37.87
1603	1.956	125044	50270	38.00
1610	2.152	131171	52615	38.72
1613	2.039	122746	49503	38.70

RESULT OF EXPERIMENT NUMBER 20

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{131}I)	Channel 2 (^{51}Cr)
Background		653	998
^{131}I Stnd		19952	20601
^{51}Cr Stnd		3093	396029
1414	1.979	20191	105943
1420	1.927	19327	101373
1425	1.917	18661	99808
1430	1.995	19027	102547
1435	1.962	18586	100706
1529	1.968	17371	102520
1536	2.067	17774	107565
1540	2.064	17855	108345
1545	2.051	17004	106243
1550	2.024	16705	104797
1555	2.028	17053	104605
1600	1.986	16973	102879
1606	2.040	16962	107020
1610	1.999	16547	105015

		Channel 1 (^{131}I)	Channel 2 (^{125}I)
Background		505	406
^{131}I Stnd		11280	3308
^{125}I Stnd		524	95200
^{51}Cr Stnd		1704	12425
1536	2.067	10076	129819
1540	2.064	10167	127096
1545	2.051	9910	123369
1550	2.024	9792	120498
1555	2.028	9916	120990
1600	1.986	9867	117319
1606	2.040	9665	118451
1610	1.999	9702	115976

RESULT OF EXPERIMENT NUMBER 21

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{51}Cr)	Channel 2 (^{125}I)
Background		16785	3483
^{51}Cr Stnd		543940	24066
Stnd. Soln.		343454	579060
10th May 1974			
1339	2.131	589976	820097
1343	2.131	575476	792545
1348	2.115	565568	767101
1353	2.116	570203	771770
1358	2.098	562677	753979
1403	2.145	570226	763345
1408	2.118	575942	754264
1413	2.094	567993	745020
16th May 1974			
1712	2.119	476793	239925
1722	2.076	471659	234943
1729	2.099	469553	234849
1733	2.053	457785	227914

RESULT OF EXPERIMENT NUMBER 22

TIMES WEIGHTS AND SUMMATED COUNTS

Time	Weight	Channel 1 (^{125}I)	Channel 2 (^{51}Cr)
Background		1532	1211
^{125}I Stnd		263754	1193
^{51}Cr Stnd		2559	25000
22nd May 1974			
1630	1.957	127254	20982
1635	1.972	125413	21034
1640	2.397	150554	24186
1645	2.209	136159	23111
1655	2.090	129824	22214
1705	1.994	107238	18648
1710	2.275	139399	23666
24th May 1974			
0753	2.160	65475	22136
0806	2.041	62431	20940
0837	2.050	61165	20633
0903	2.059	61730	21349
0924	2.085	60926	21036
0937	2.088	60637	21564
0951	2.119	61692	22337
1000	2.086	60023	21910
		Channel 1 (^{131}I)	Channel 2 (^{51}Cr)
Background		3239	2660
^{125}I Stnd		30171	90436
^{51}Cr Stnd		3826	121691
1008	2.078	29572	136048
1003	2.086	3133	50913
1013	2.083	28912	134675
1018	2.092	28817	132377
1028	2.131	28357	133953
1038	2.074	27240	128912
1049	2.098	26835	128632
1110	2.085	26393	125979
1335	2.114	21481	105961

RESULT OF EXPERIMENT NUMBER 23

TIMES WEIGHTS AND SUMMATED COUNTS

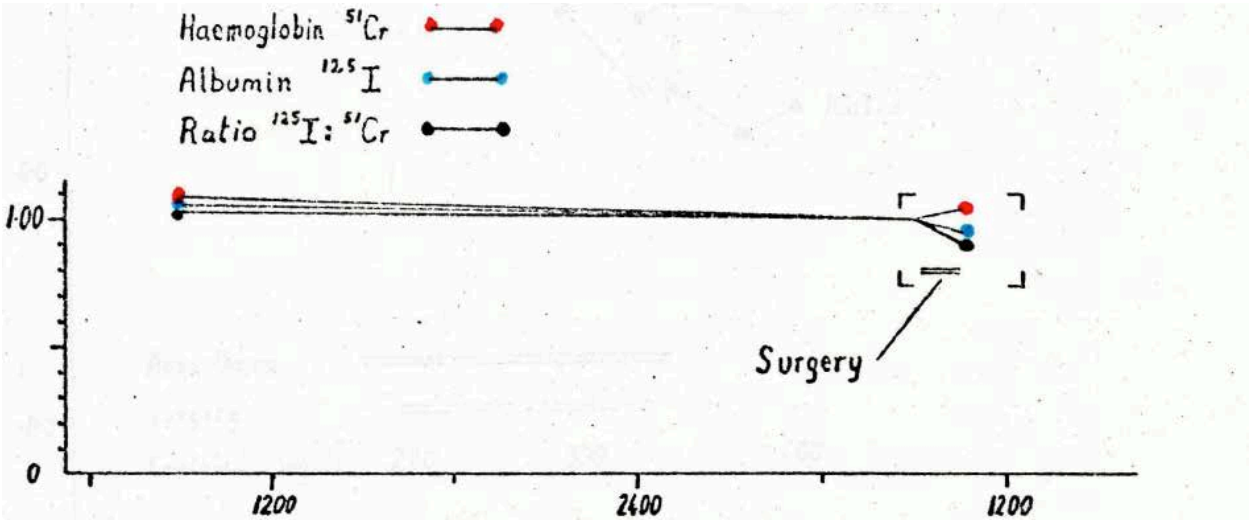
Time	Weight	Channel 1 (^{125}I)	Channel 2 (^{51}Cr)
Background		1399	2902
^{125}I Stnd		147310	3001
^{51}Cr Stnd		9889	154678
12th June 1974			
1517	2.139	83462	109631
1522	2.116	82140	105728
1525	2.096	80717	105727
1528	2.031	77648	101297
1538	2.083	78386	103016
1544	2.088	77999	103348
1552	2.067	76570	103001
1512	1.940	84105	63635
14th June 1974			
0805	2.097	41680	103147
0825	2.083	40166	98761
0905	2.074	40680	102620
0920	2.072	40478	101958
0947	2.072	40765	107082
1008	2.051	39088	102919

		Channel 1 (^{131}I)	Channel 2 (^{51}Cr)
Background		1497	400
^{131}I Stnd		130170	80621
^{51}Cr Stnd		2723	39004
1023	2.071	52753	92381
1008	2.051	2239	27581
1025	2.066	52456	91811
1029	2.023	51160	89786
1037	2.058	42525	73456
1048	2.055	45394	77187
1053	2.054	42482	73015
1057	2.075	41302	73102
1102	2.069	37337	63864
1130	2.083	31032	54088
1220	2.046	31549	56923
1013	1.934	59447	66102

3. Degree of Extravasation of Albumin relative to Haemoglobin - D.E.A.R.T.H. Graphs

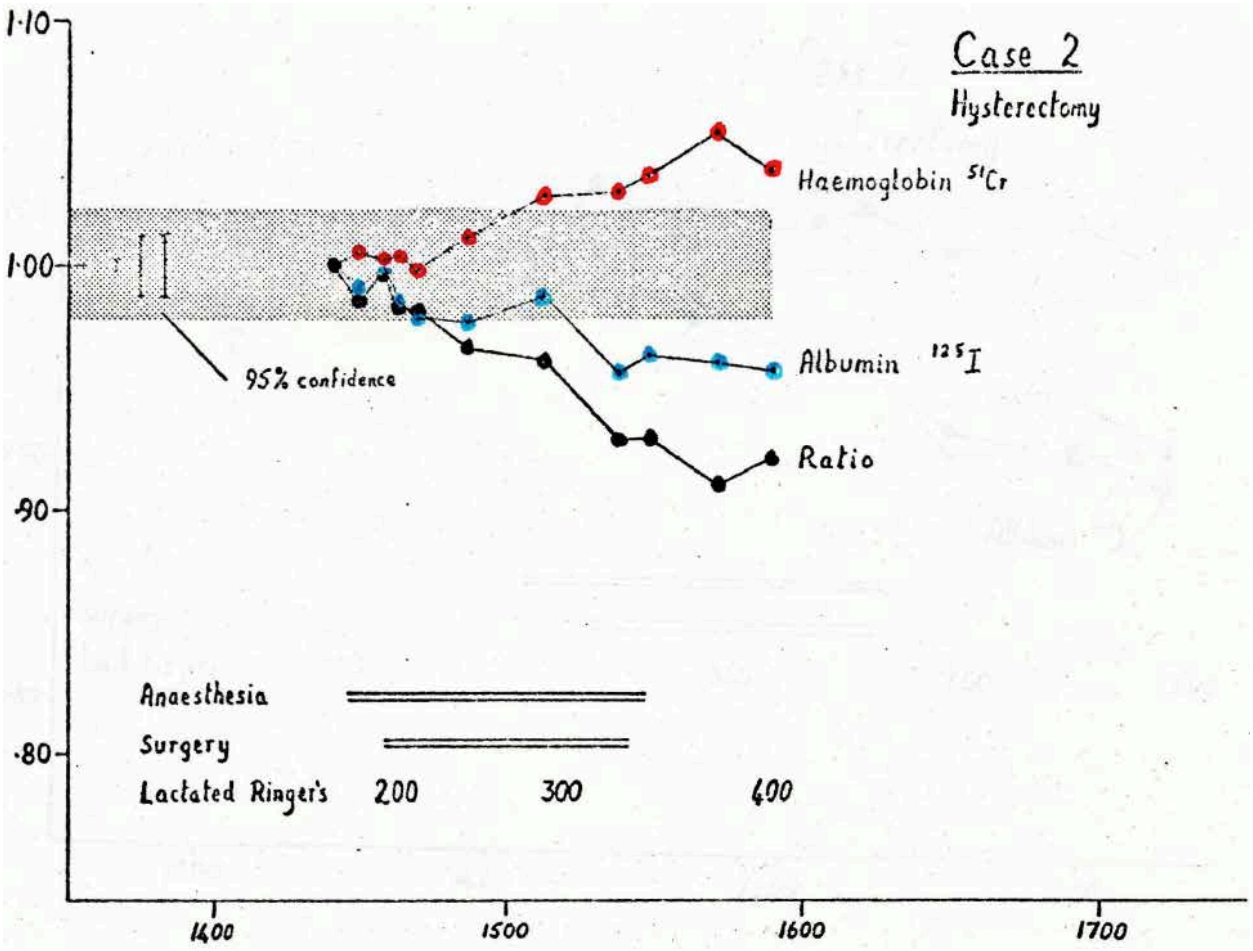
Each graph on the following pages shows the change in albumin radio activity, in haemoglobin radio activity and in relative ratio, and also shows the times of other events and the 95% confidence limits (see Method sections 8, 141 17 and 21).

The preliminary graph on the next page indicates the scale used as well as the magnitude of the changes under normal conditions during twenty-four hours.



Graph showing approximate daily change in relative blood levels of ^{51}Cr and ^{125}I and in their relative ratio.

Inset shows area and scale of the results which follow and the type of change encountered.

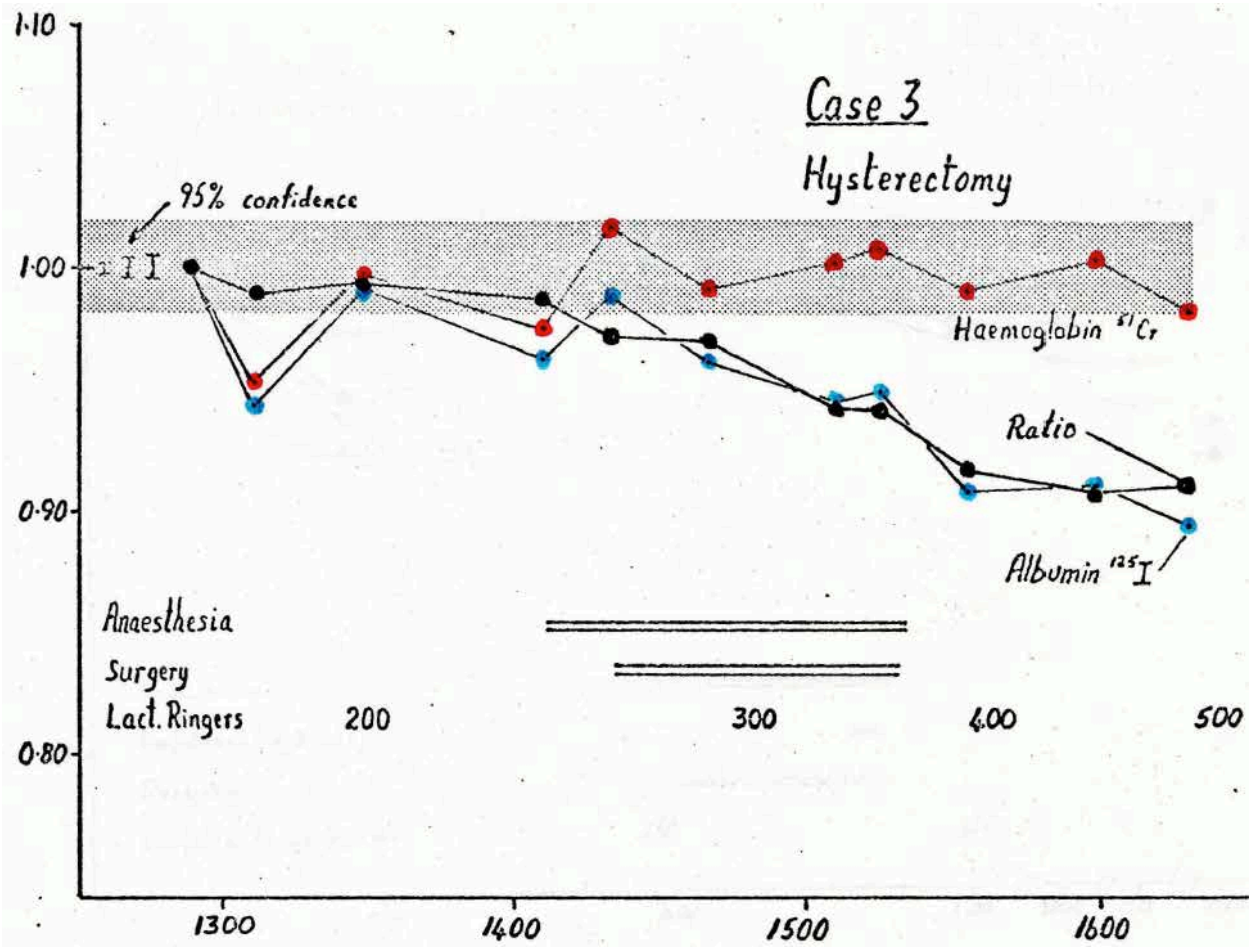


Mr. J.K. Age 55. 95% confidence ±0.0226.

	Relative ratio	D.E.A.R.T.H.
68 minutes after incision	0.9104	0.0896

Haemoglobin shows marked haemoconcentration despite intravenous infusion.

(Relative haemoglobin count 1.0541 68 mins. after incision.)

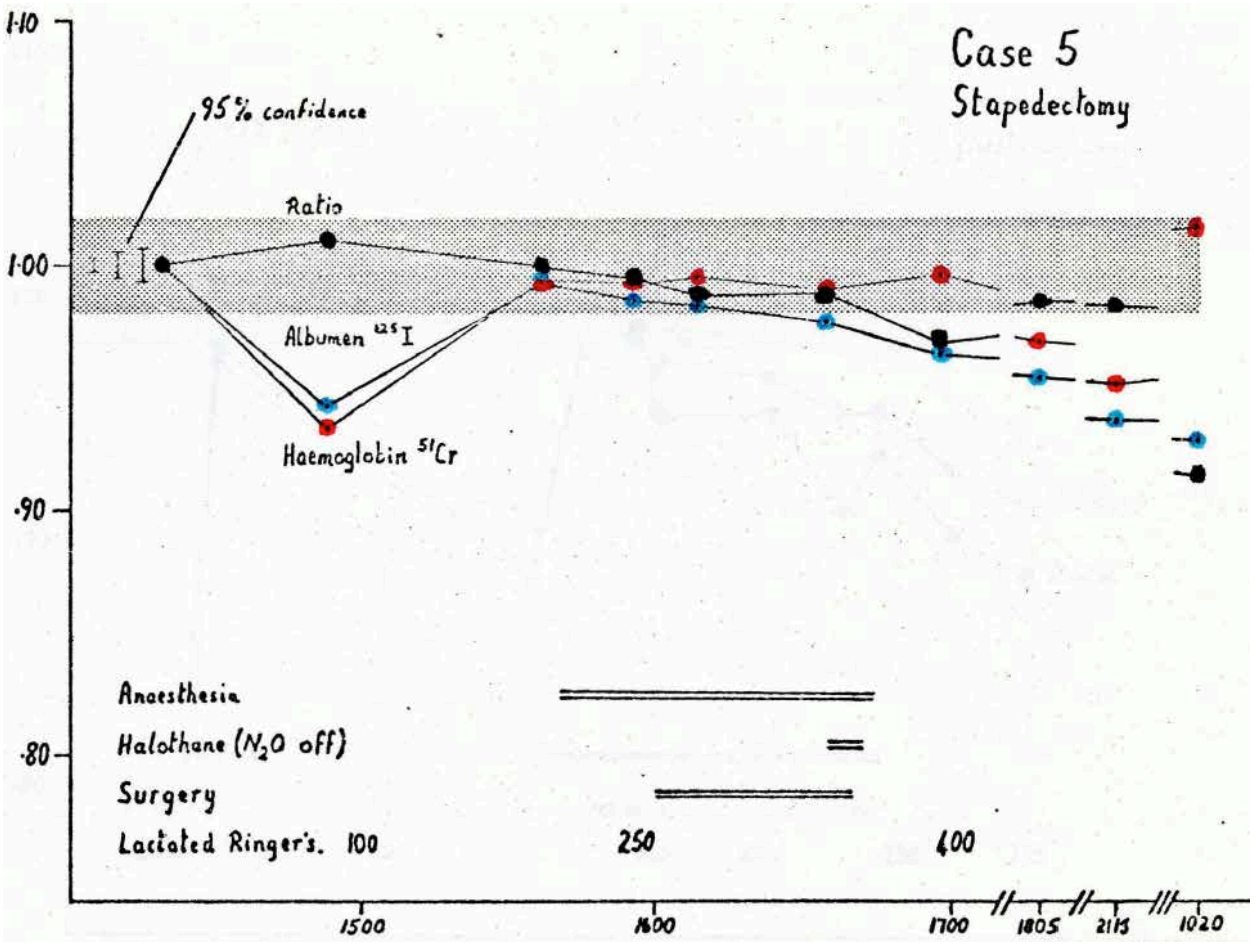


Mrs. G.W. Age 41. 95% confidence +0.0194.

	Relative ratio	D.E.A.R.T.H.
After 73 minutes pre-anaesthetic	0.9864.	0.0136
98 minutes after incision	0.9074	0.0926

1306 sample shows effect of contamination with I.V. fluid.

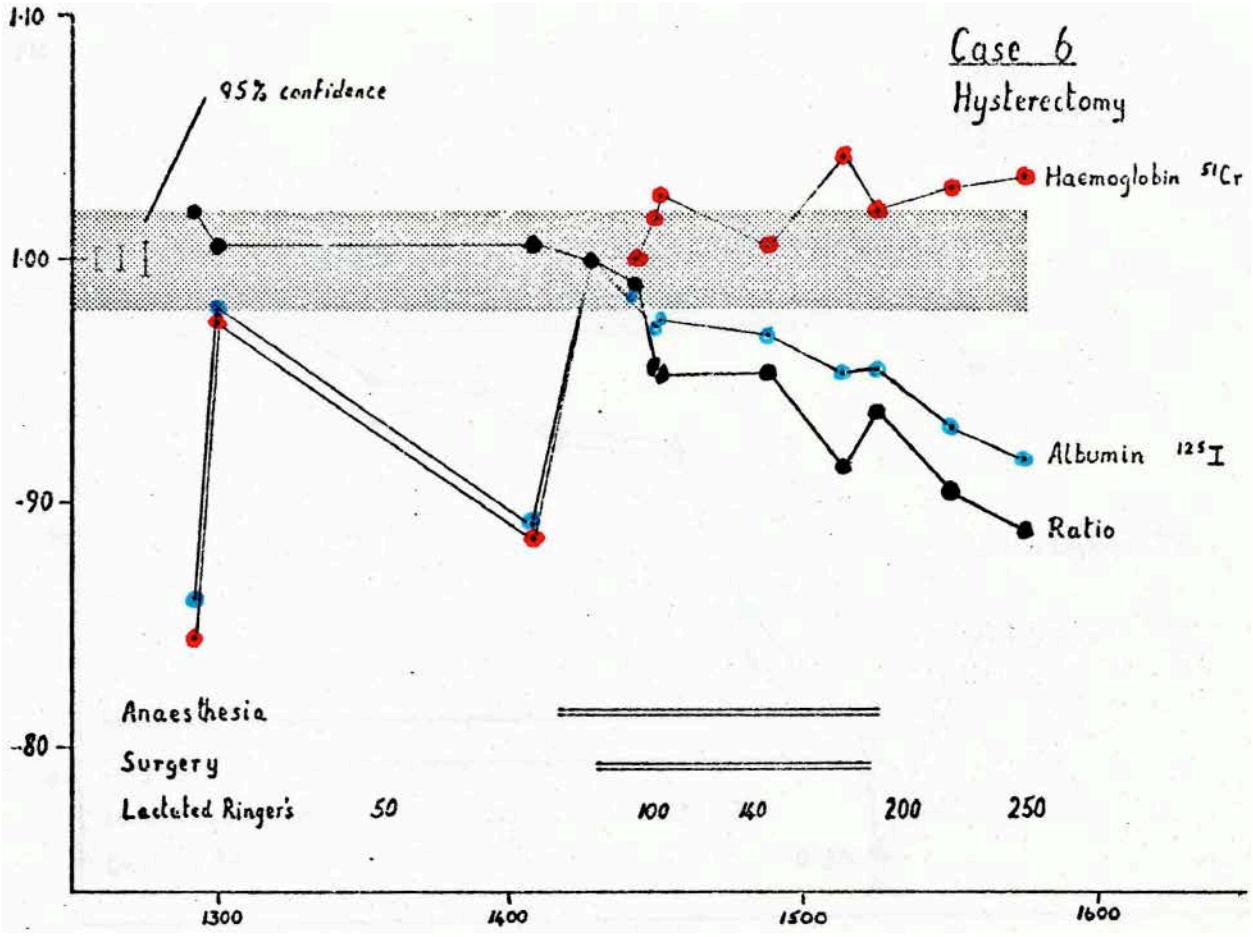
Haemoglobin shows evidence of haemodilution prior to incision but following incision shows no evidence of dilution despite infusion.



Mrs. O.T. Age 62. 95% confidence ±0.0199.

	Relative ratio	D.E.A.R.T.H.
After 79 minutes pre-anaesthetic	0.9991	.0009
54 mins. after induction	0.9879	.0121
Emerging from anaesthesia	0.9679	.0321
Next morning (1020)	0.9138	.0862

1453 sample shows effect of contamination with I.V. fluid.



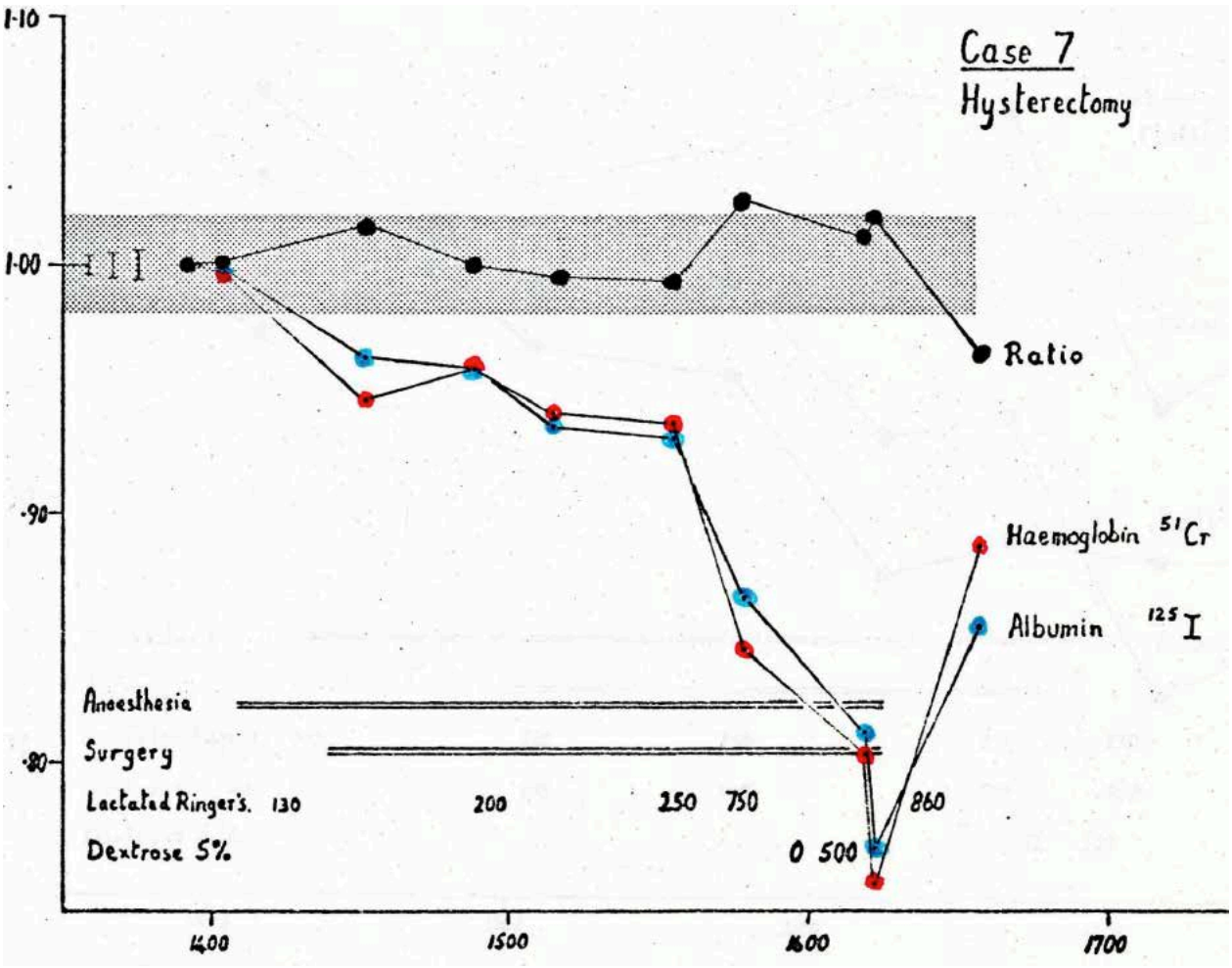
Mrs. D. W. Age 42. 95 confidence ±0.0199.

In this experiment the pre-operative values were subject to dilution and therefore the first operative value was used for reference.

	Relative ratio	D.E.A.R.T.H.
Initial value (1255)	1.0196	-
70 minutes later	1.0062	0.0131
86 minutes after incision	0.8879	0.1291

1255 and 1405 samples show effect of contamination with I. V. fluid.

Haemoglobin shows haemoconcentration despite I.V. infusion.



Mrs. J.K. Age 38. 95 % confidence ±0.0198.

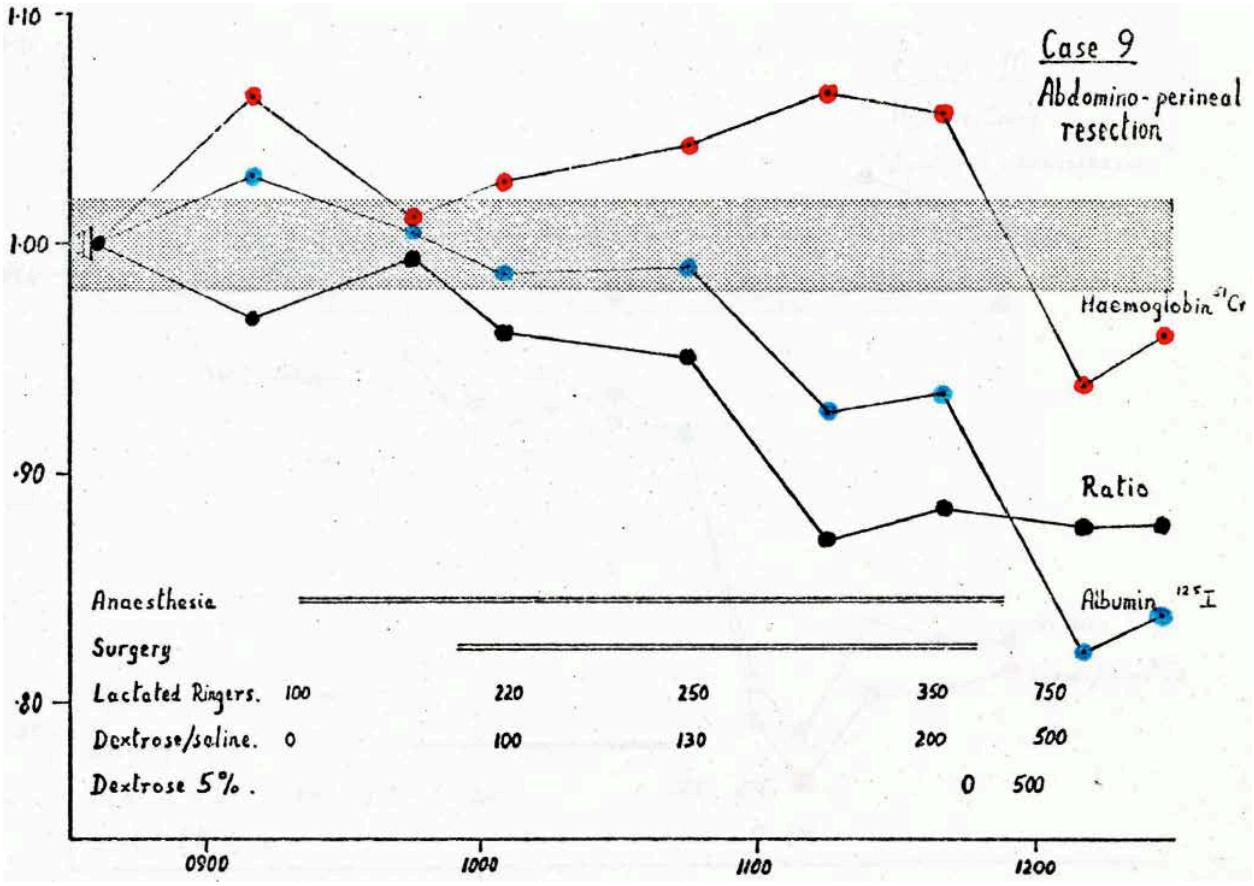
First point is mean value for 4 samples,

the second and final are mean values for 3 samples.

Other points are mean values for 2 samples.

	Relative ratio	D.E.A.R.T.H.
After first 37 minutes	1.017	
131 minutes after incision	0.964	0.036

Effects of rapid intravenous infusion demonstrated



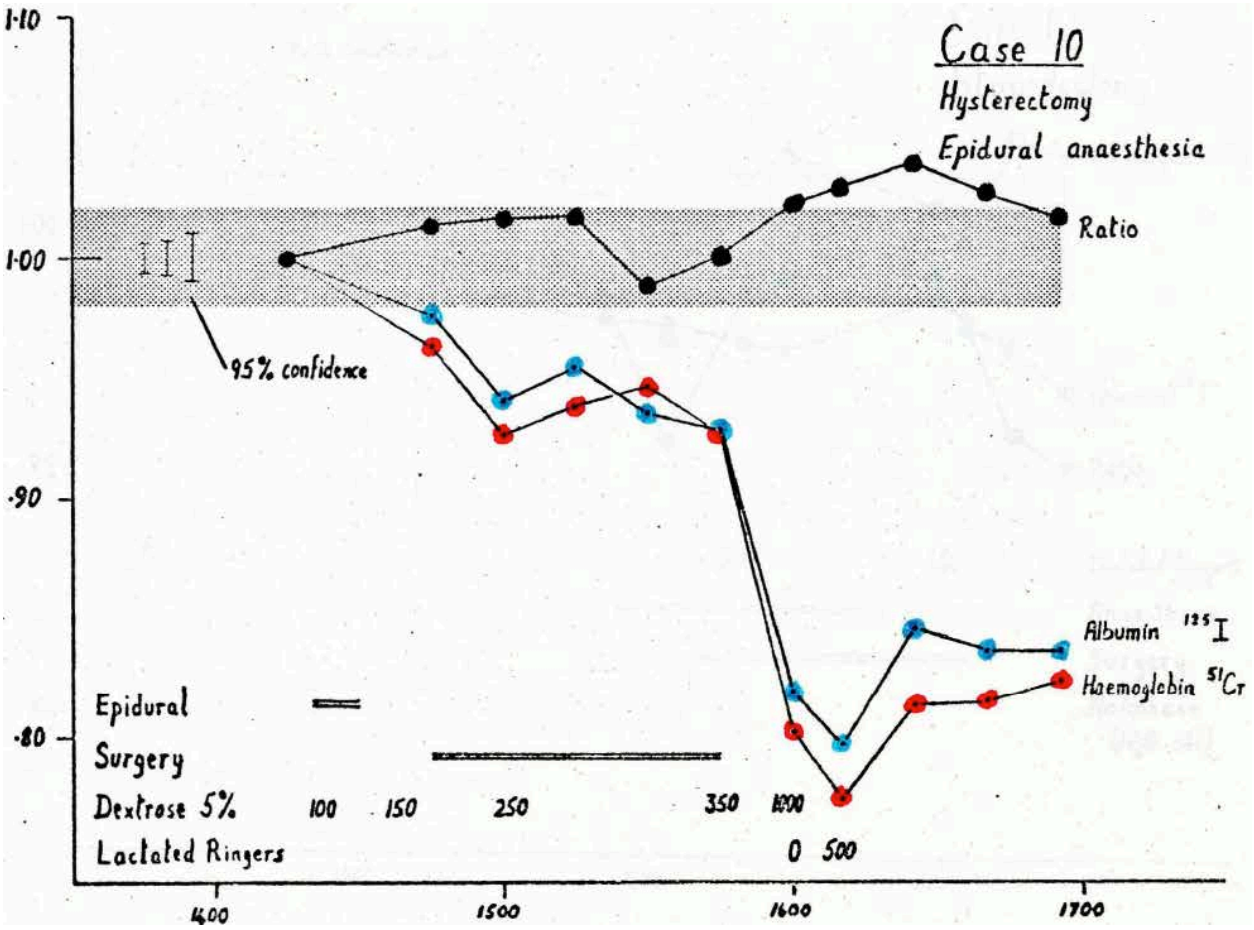
Mr. F.S. Age 60. 95% confidence ±0.0203.

First point is mean for 4 samples,

and the final point is mean for 4 samples.

	Relative ratio	D.E.A.R.T.H.
After 70 minute period before incision	0.994	0.006
One reading during the 70 minutes	0.968	0.032
80 minutes after incision	0.870	0.130

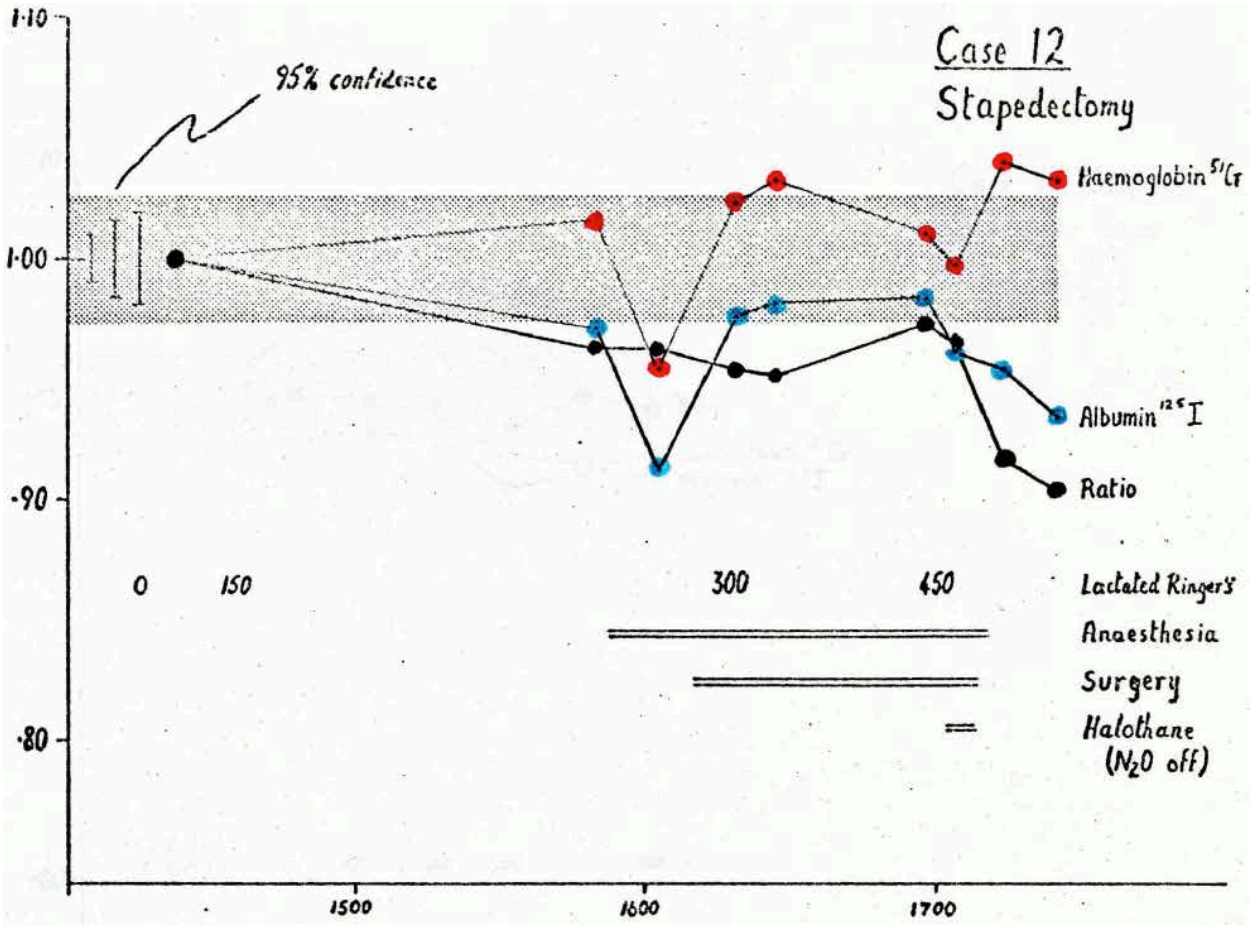
Haemoglobin shows haemoconcentration despite infusions.



Mrs. J.W. Age 47. 95% confidence ±0.0212

Initial value is mean value for 4 samples.

	Relative ratio	D.E.A.R.T.H.
During initial 30 minutes (incl. epidural):	1.013	-
60 minutes after incision	1.001	-
Post-op: After I.V. Dextrose (1610)	1.029	-
After I.V. Ring. Lact. (1625)	1.039	-

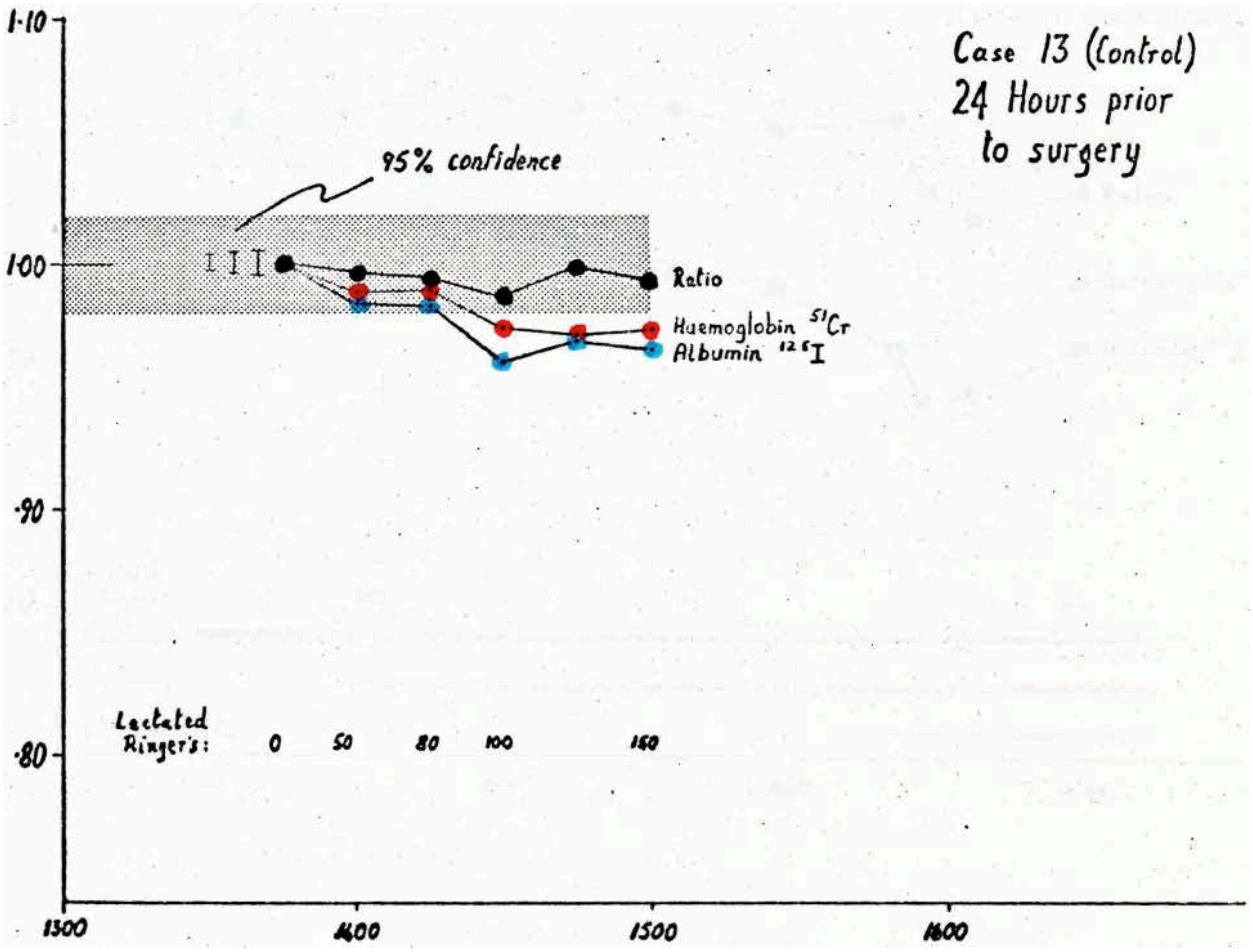


Mr. R.S. Age 45. 95% confidence ±0.0268.

Initial point is mean value for 4 samples.

	Relative ratio	D.E.A.R.T.H.
During initial 88 minutes (pre-anaes.)	0.963	0.037
59 minutes after incision	0.964	0.036
63 minutes after incision extubated		
69 minutes after incision	0.918	0.082
80 minutes after incision	0.904	0.906

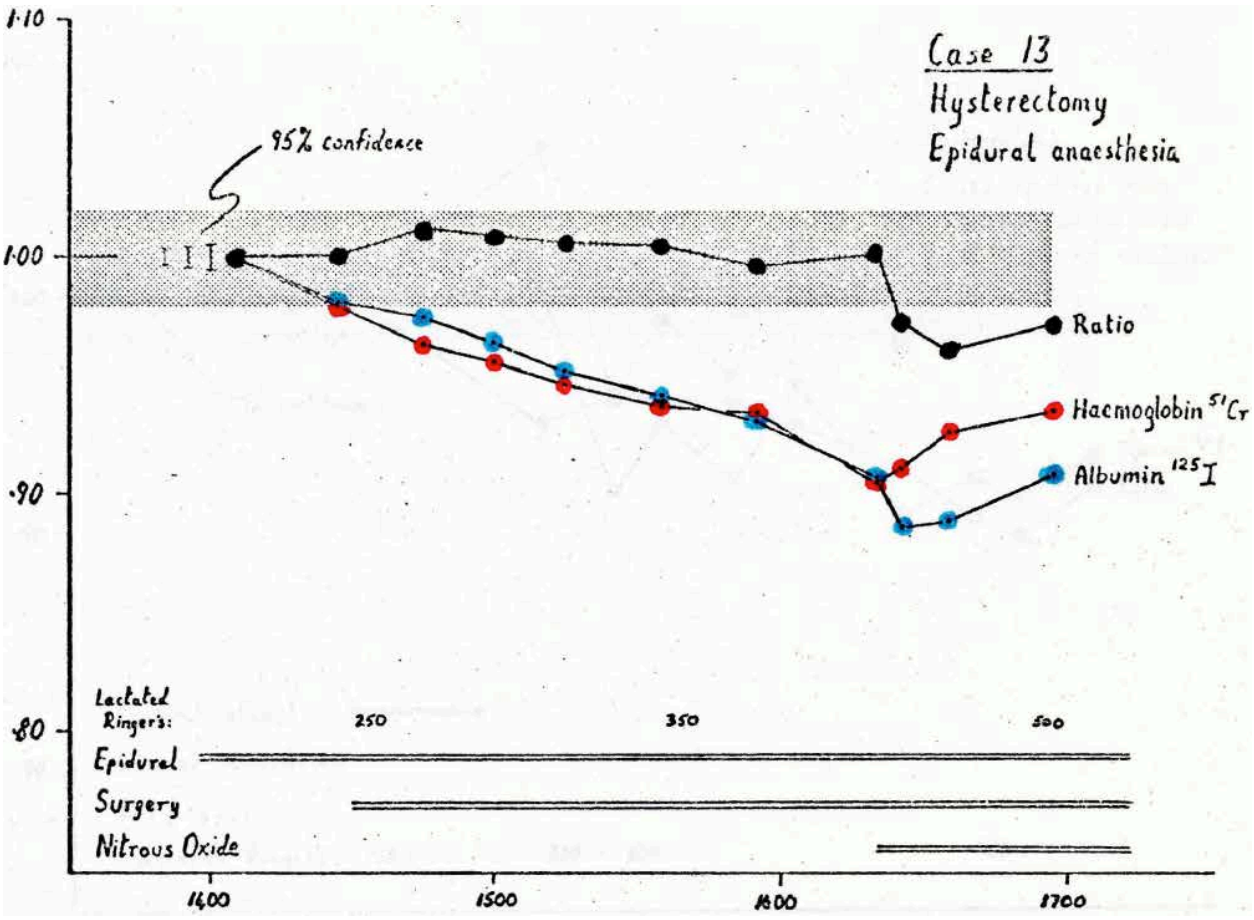
Haemoglobin shows haemoconcentration despite infusion.



Miss M.C. Age 56. 95% confidence ±0.0196.

Initial point is mean value for 4 samples.

During 75 minutes no significant D.E.A.R.T.H.



Miss M.C. Age 56. 95% confidence ±0.0196.

Initial point is mean value £or 4 samples.

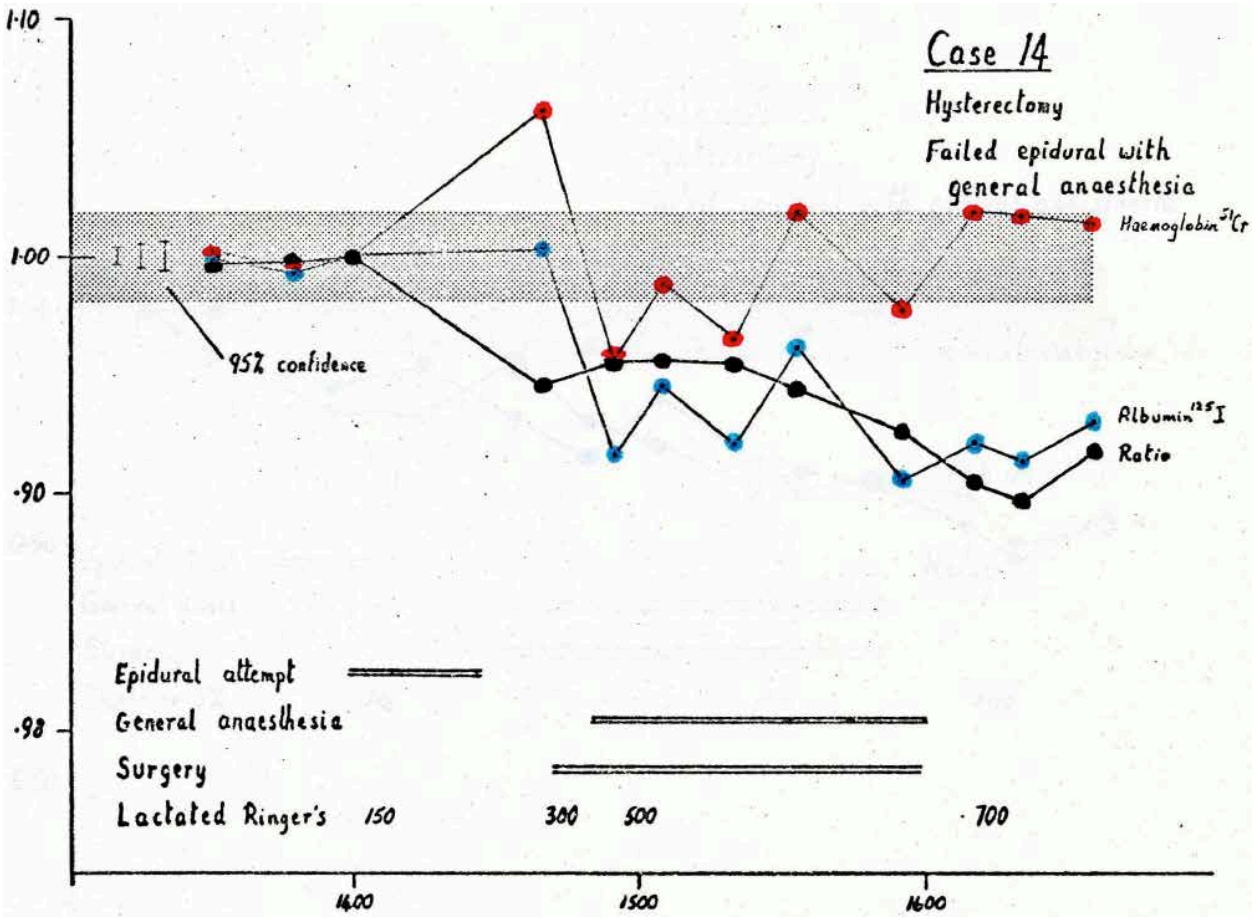
During 135 minutes including 115 minutes surgery,

no significant D.E.A.R.T.H. (relative ratio = 1.002).

At this time patient distressed by pain, N_2O started:

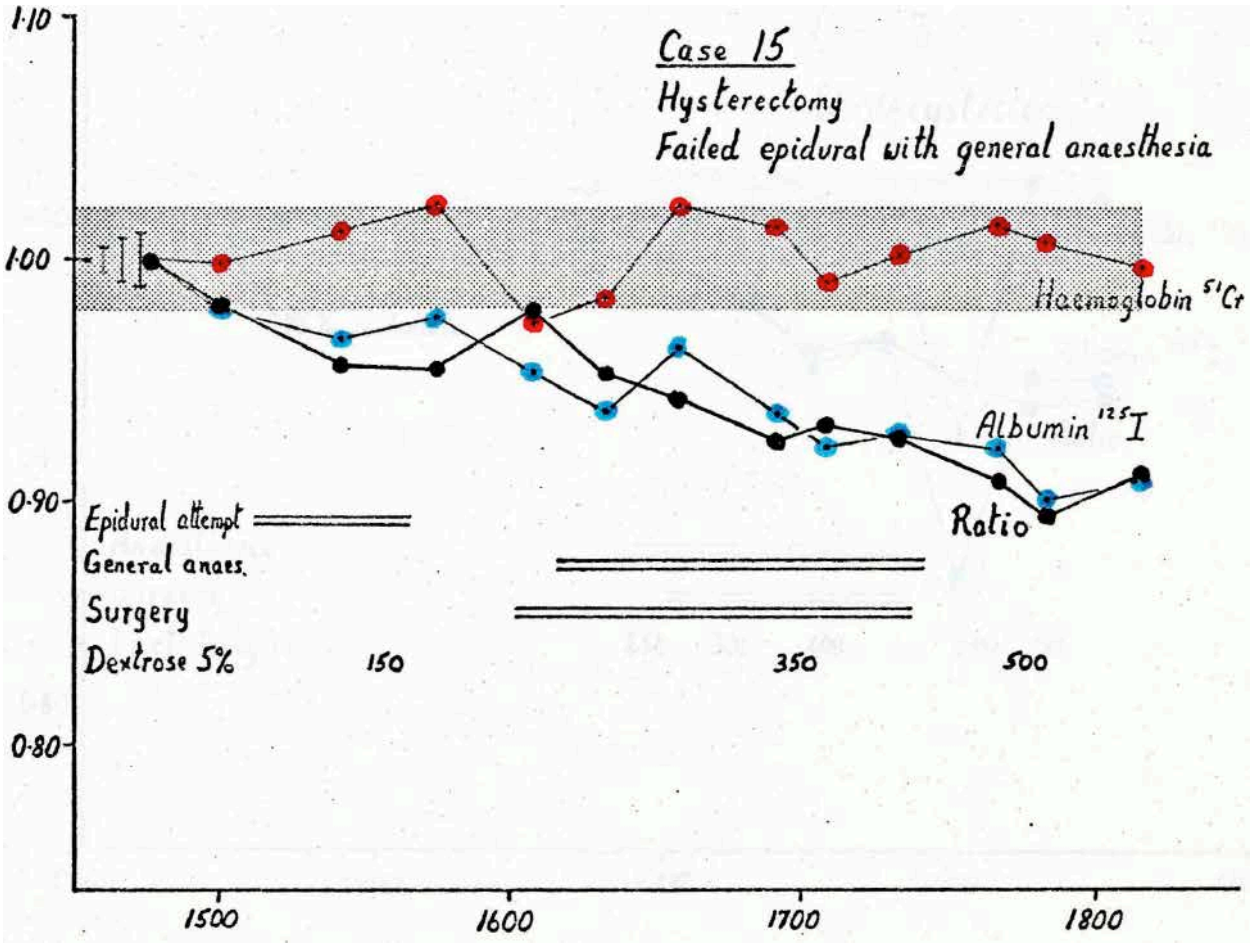
	Relative ratio	D.E.A.R.T.H.
15 minutes after pain	0.960	0.0140

Not plotted: At 12.50 a.m. (75 minutes before first value on graph) relative ratio was 1.023. Thus, during transport and epidural, D.E.A.R.T.H. = 0.023.



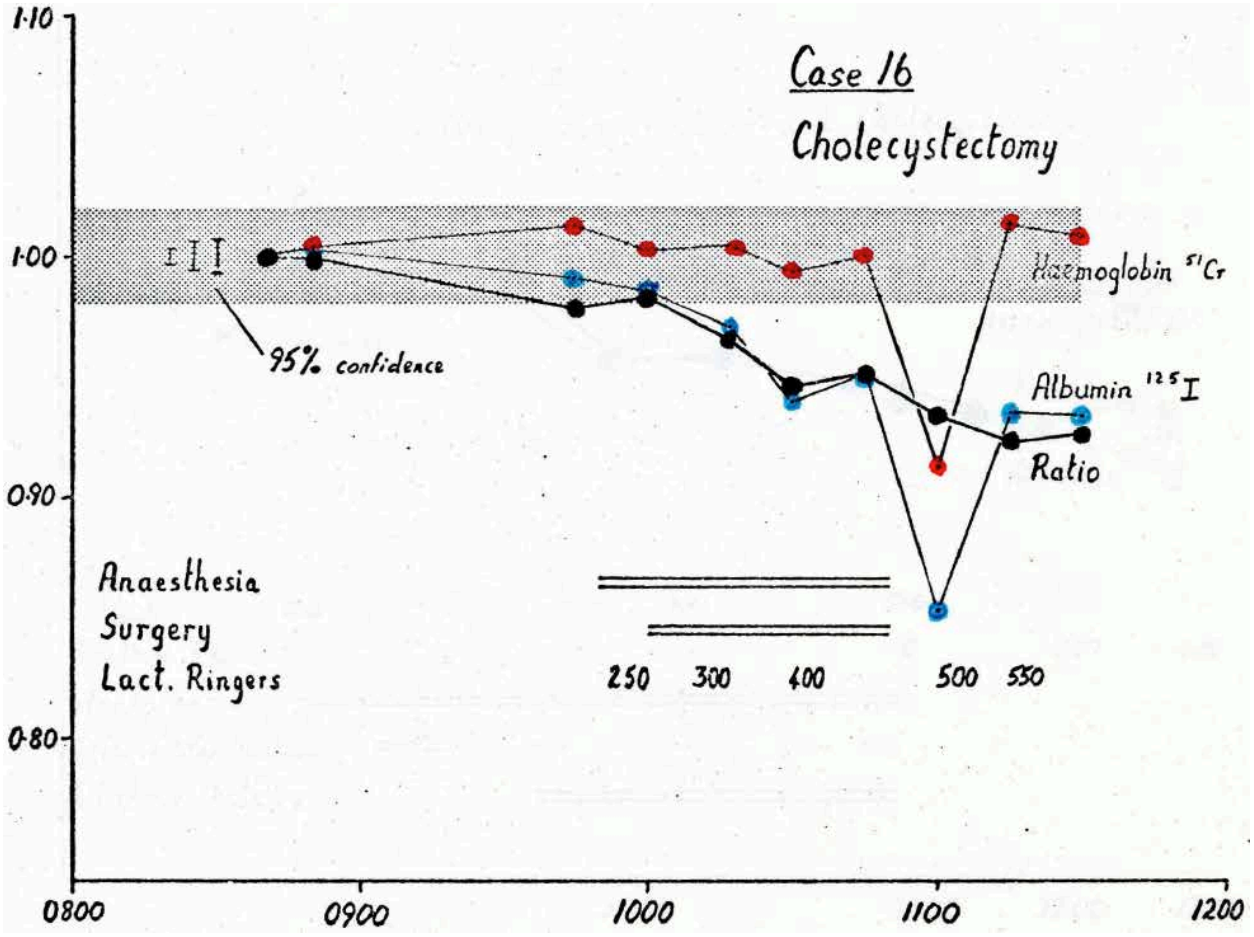
Mrs. L.D. Age 45. 95% confidence ±0.0199.

	Relative ratio	D.E.A.R.T.H.
After 30 mins. (prior to epidural attempt)	1.003	-
After next 40 mins. (incl. epidural attempt)	0.946	.054
110 minutes after incision	0.897	.104



Mrs. P.B. Age 31. 95% confidence +0.0215.

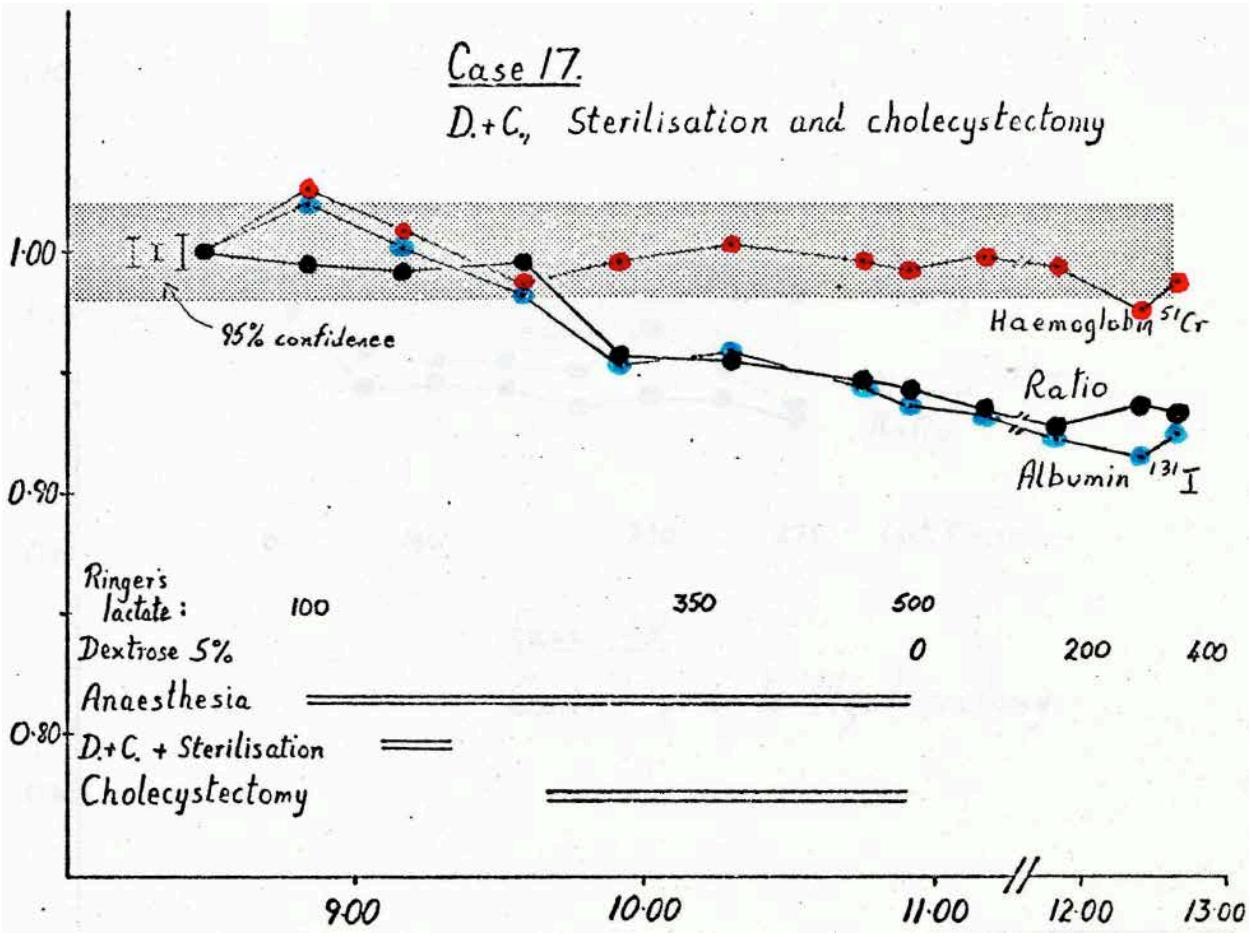
	Relative ratio	D.E.A.R.T.H.
After 15 mins. prior to epidural attempt	0.980	0.020
After 15 mins. of epidural attempt	0.956	0.014
108 mins. after incision	0.89	0.11



Mrs. W.G. Age 70. 95% confidence ±0.0199.

	Relative ratio	D.E.A.R.T.H.
After 80 mins. prior to incision	0.983	0.017
having fallen at 65 mins. To	0.978	0.022
75 mins. after incision	0.923	0.077

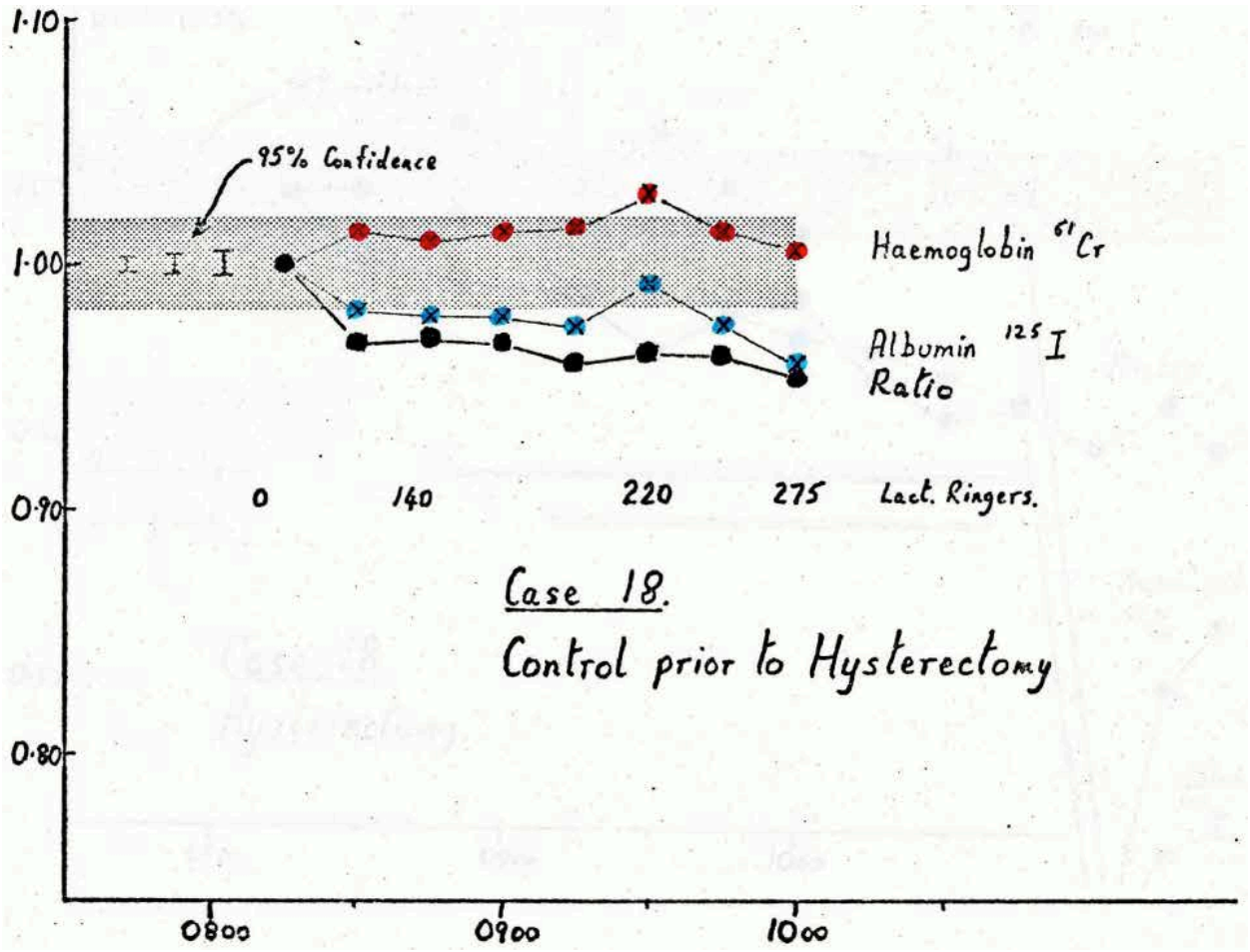
1100 sample shows effect of contamination with I. V. fluid.



Mrs. E.U. Age 39. 95% confidence ±0.0201.

Each point is mean value for 2 samples.

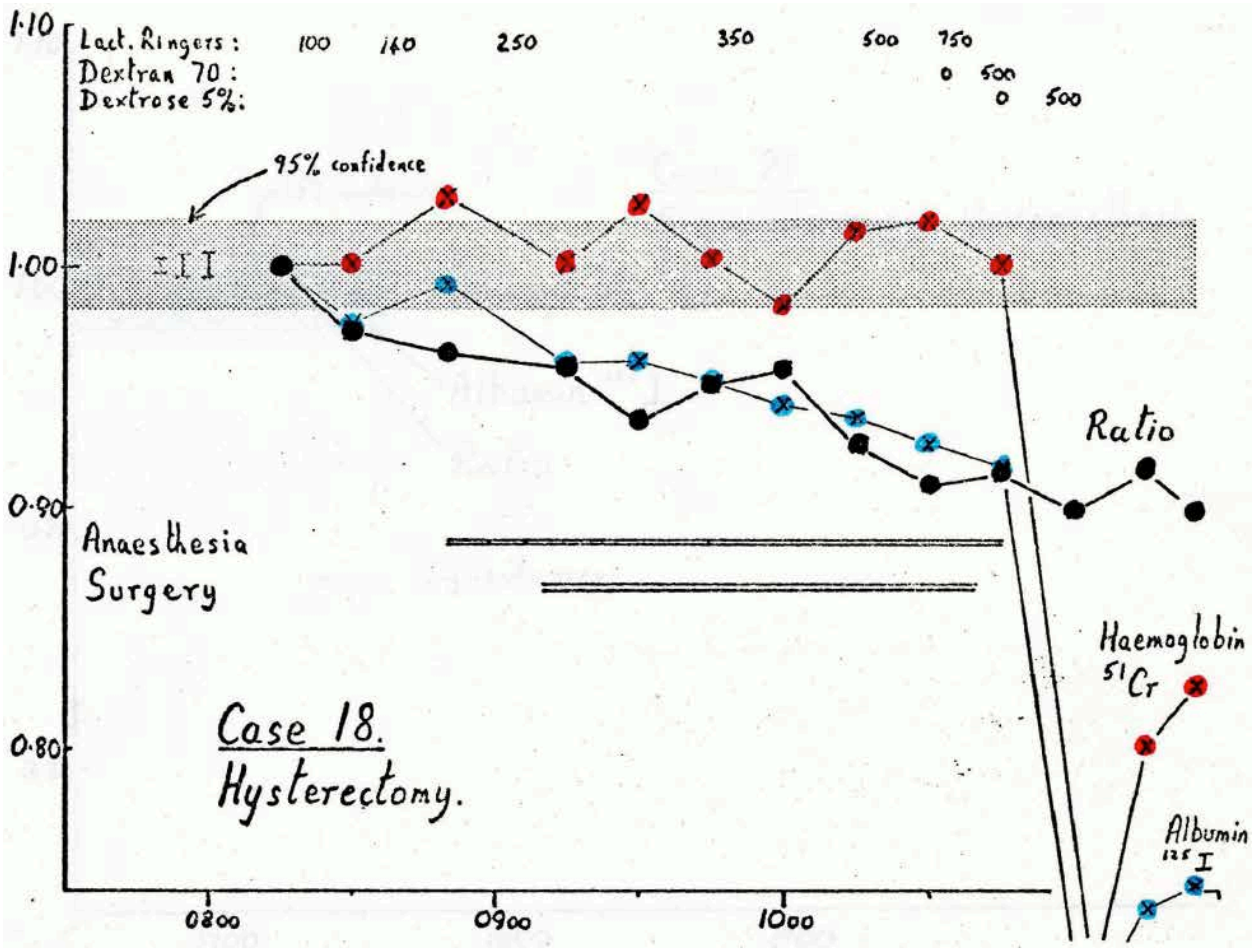
	Relative ratio	D.E.A.R.T.H.
After 67 mins. (incl. induction, dilation and curettage, and sterilisation)	0.996	0.004
15 mins. after main 1ncision	0.956	0.044
130 mins. after incision	0.928	0.072



Mrs. E.C. Age 39. 95% confidence ±0.0194.

3rd April 1974.

	Relative ratio	D.E.A.R.T.H.
After 15 minutes	0.968	0.032
After next 90 minutes	0.954	0.046

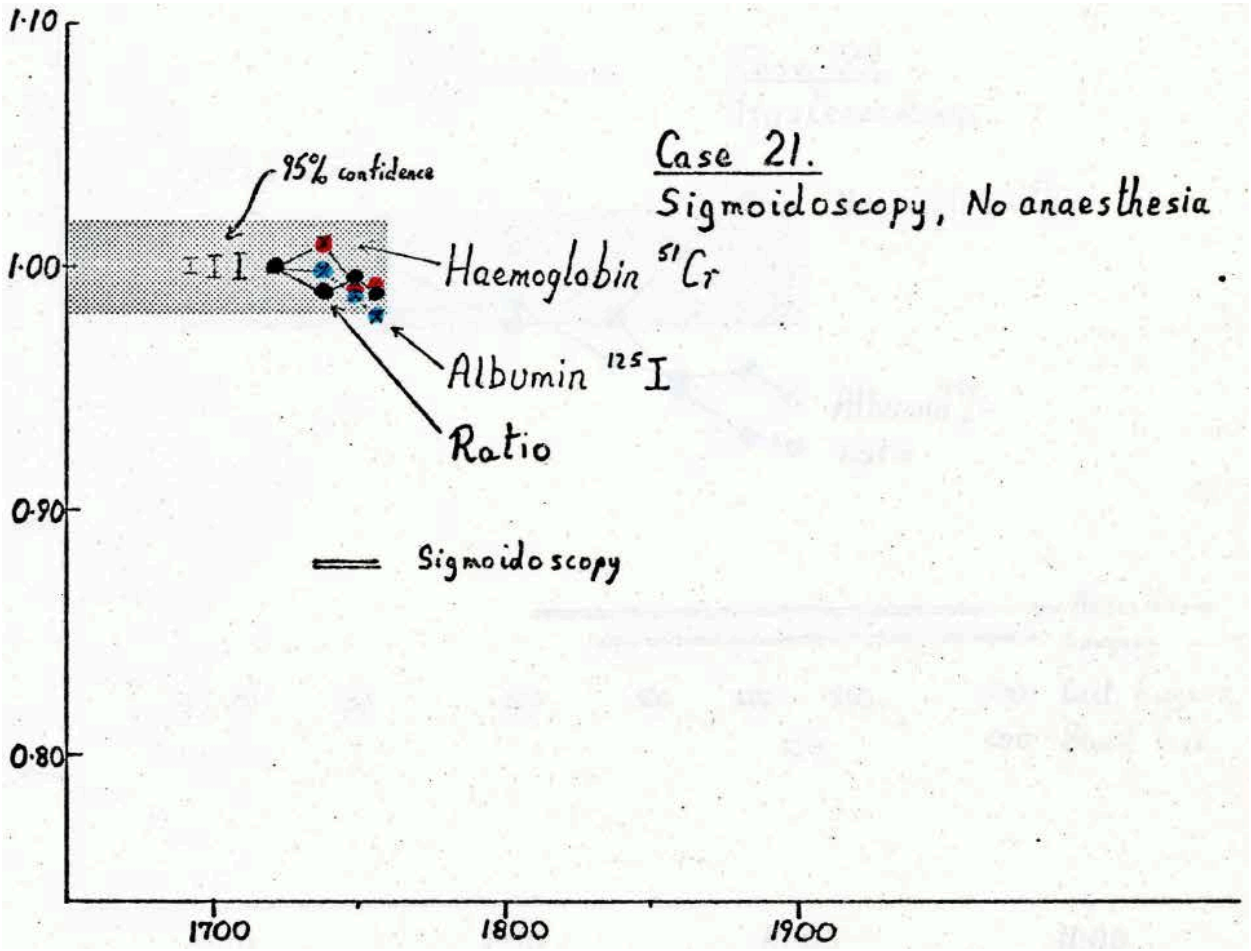


Mrs. E. C. Age 39. 95% confidence ±0.0195.

4th April 1974

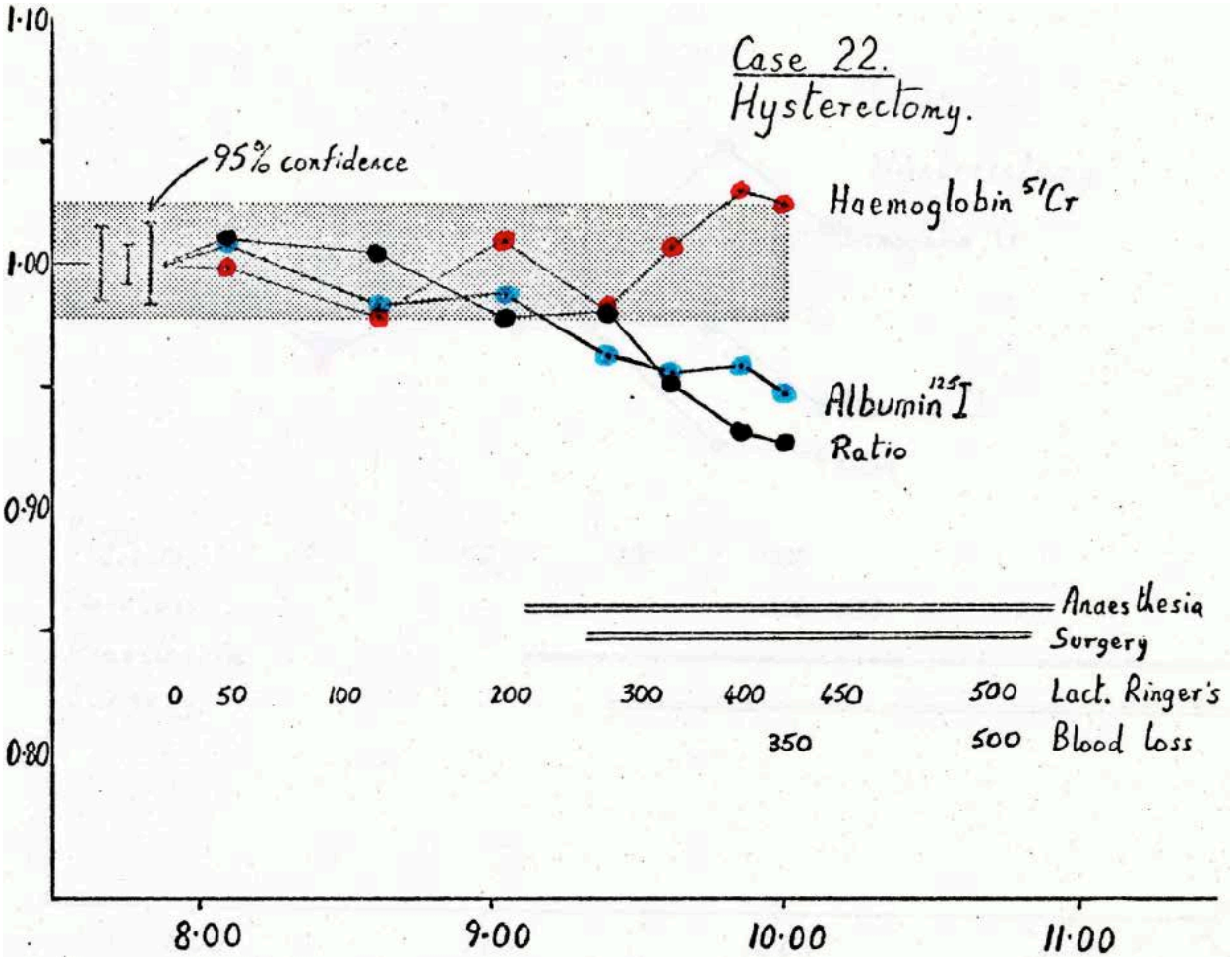
	Relative ratio	D.E.A.R.T.H.
After 15 minutes	0.974	0.026
After next 20 minutes	0.964	0.036
110 minutes after incision	0.898	0.102

1100 sample shows effect of rapid infusion.



Mrs. B.J. Age 73. 95% confidence ±0.0195.

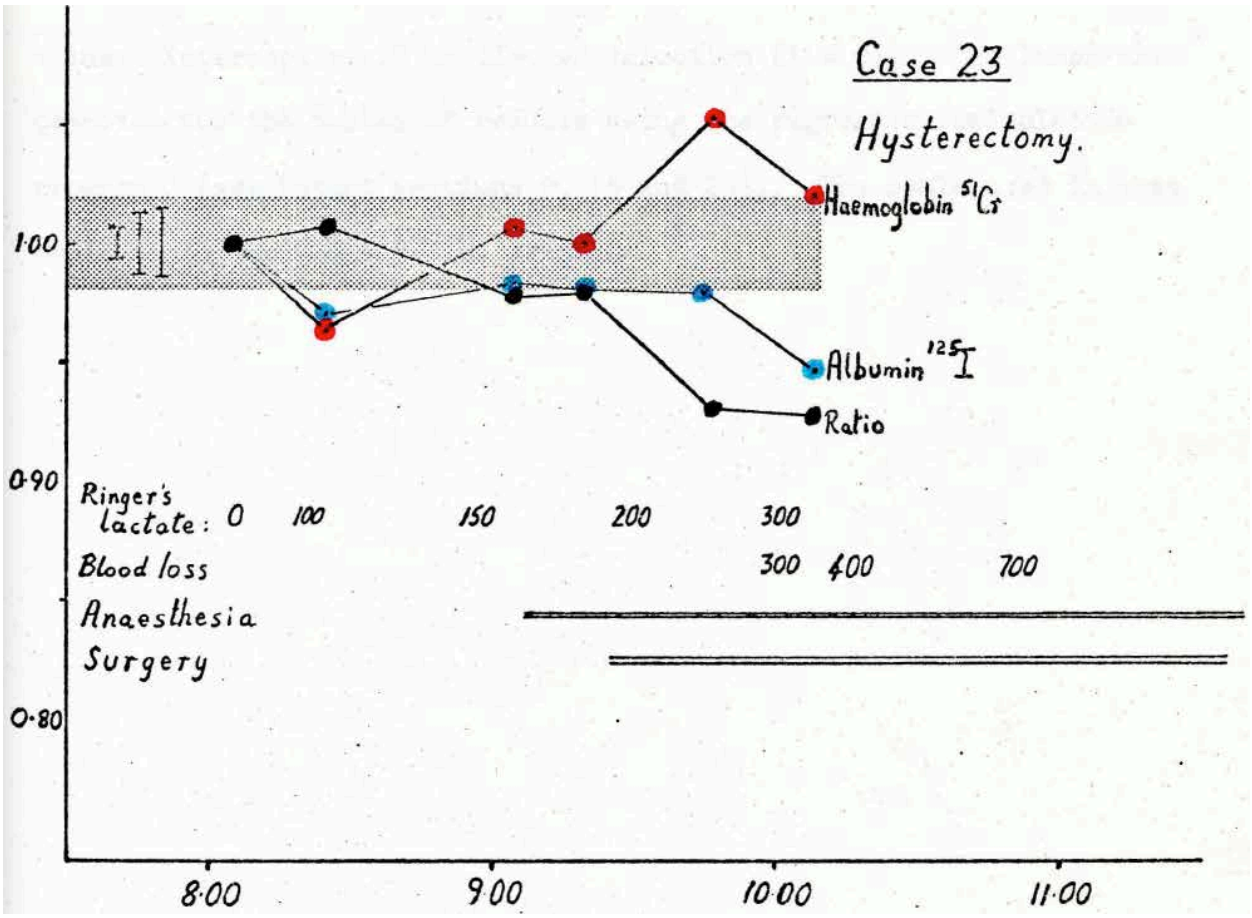
	Relative ratio	D.E.A.R.T.H.
After 21 minutes	0.989	0.011



Mrs. R.M. Age 43. 95% confidence ±0.0250.

	Relative ratio	D.E.A.R.T.H.
After 70 mins. prior to anaesthetic	0.980	0.020
40 mins. after incision	0.924	0.076

Haemoglobin shows haemoconcentration despite infusion.



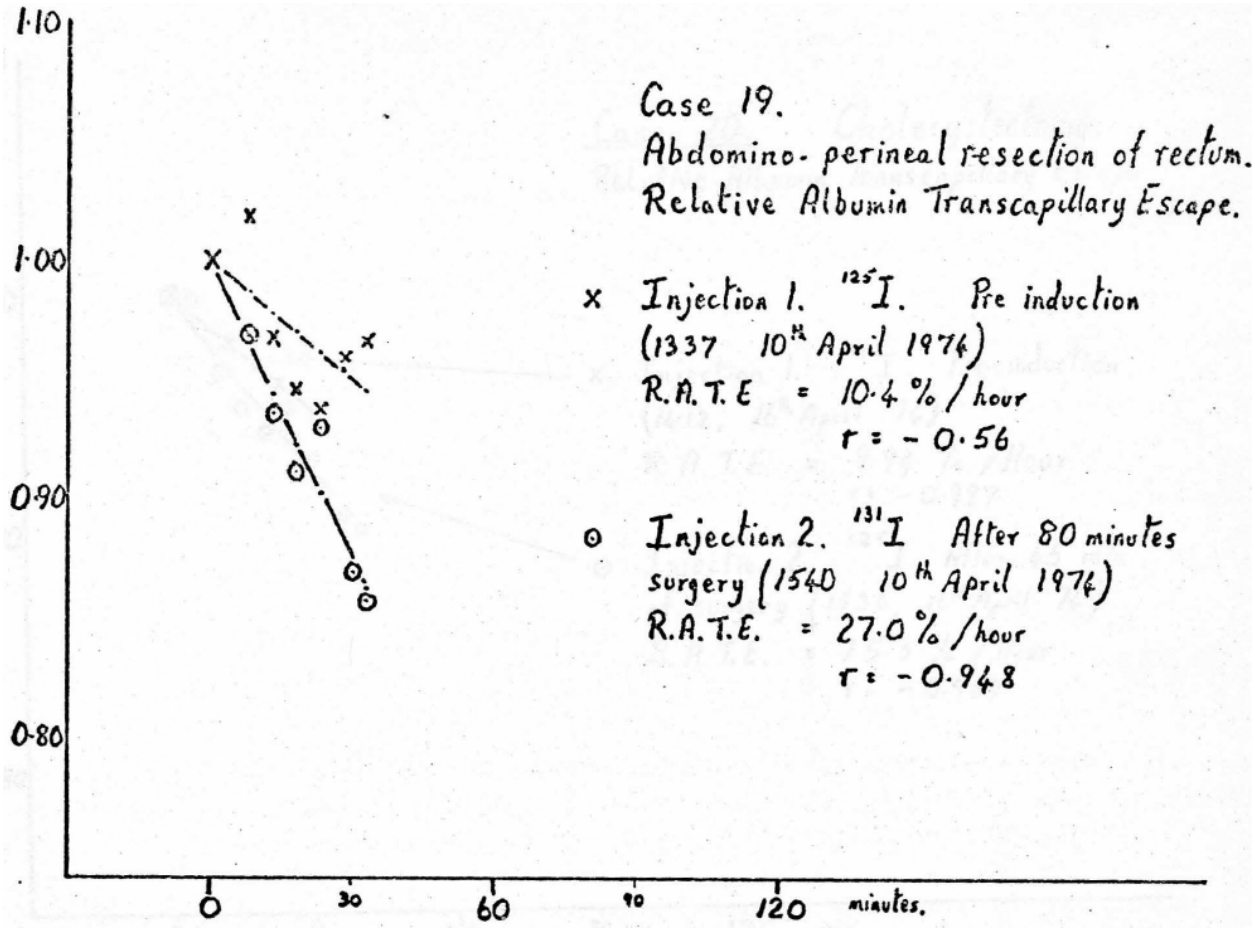
Mrs. P.E. Age 44. 95% confidence ±0.0202.

	Relative ratio	D.E.A.R.T.H.
After 60 mins. prior to anaesthetic	0.977	0.023
43 mins. after incision	0.928	0.072

Haemoglobin shows haemoconcentration despite infusion.

4. Relative Albumin Transcapillary Escape - R.A.T.E. graphs.

Each graph shows the values of the relative ratio, scaled to the value: intercept = 1.0 at time of injection (t = 0). The lines are computed for the tables of results using the regression calculation program C (see Method sections 9, 15 and 23). The scale used is that employed in the D.E.A.R.T.H. graphs.

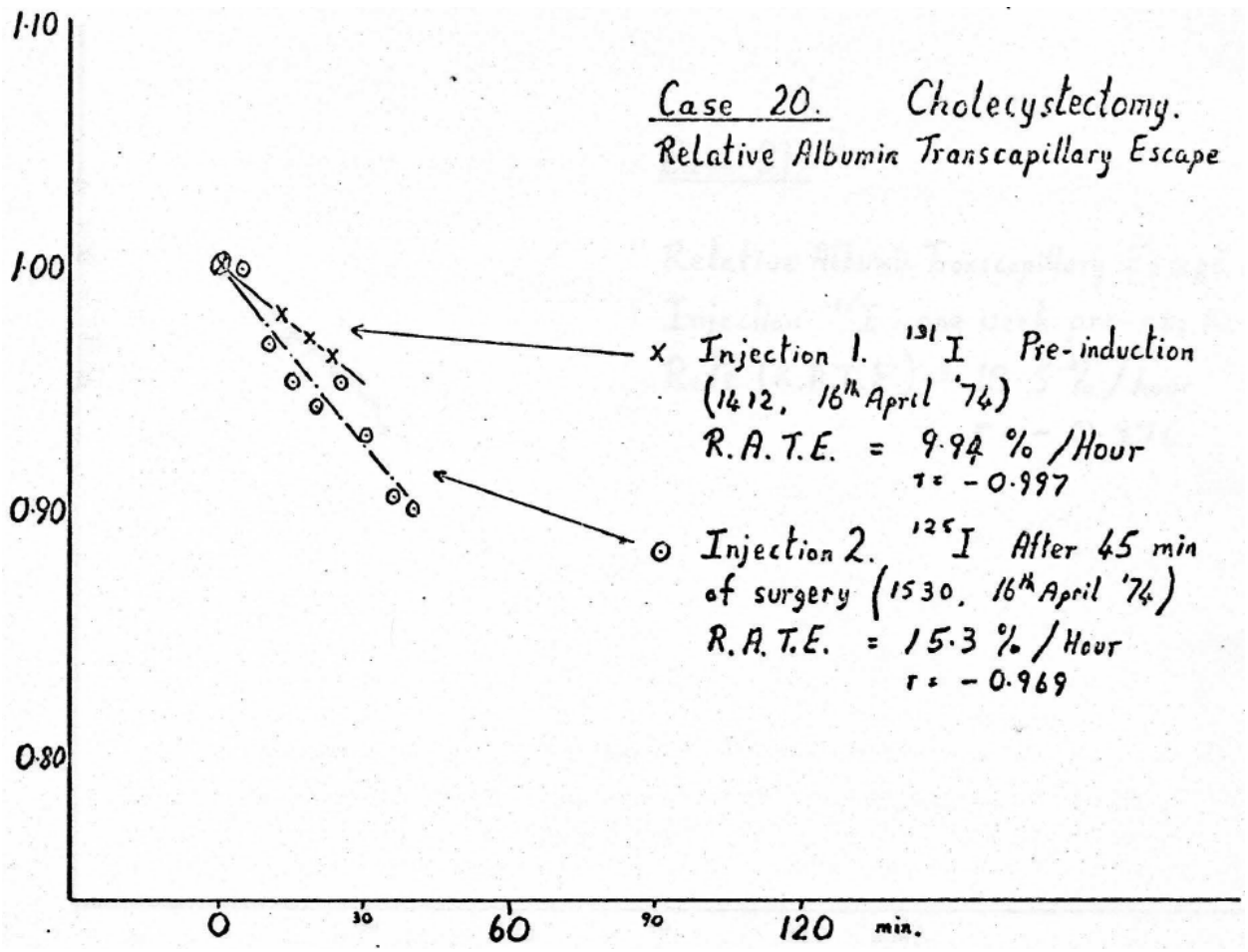


Mr. D.E. Ago 74. Anaesthesia commenced immediately following first series of samples taken.

I.V. Fluids. Lactated Ringer's solution, less than 100 ml. during the first measurement. After 88 minutes of anaesthesia: Lactated Ringer's solution 450 ml., Blood 800 El., Dextran 70 in 5% Dextrose 500. Then during second measurement, Lactated Ringer's 50 ml. and Blood 25 ml.

(Samples taken at 4 minutes were omitted in calculating R.A.T.E. in both graphs because of evidence of inadequate mixing within such short periods.)

Both graphs derived by regressing log (radio-iodine activity per gram/ haematocrit) against time. See Method 9c. The rise in haematocrit during the second measurement accounts for approximately 7% per hour of the measured change.



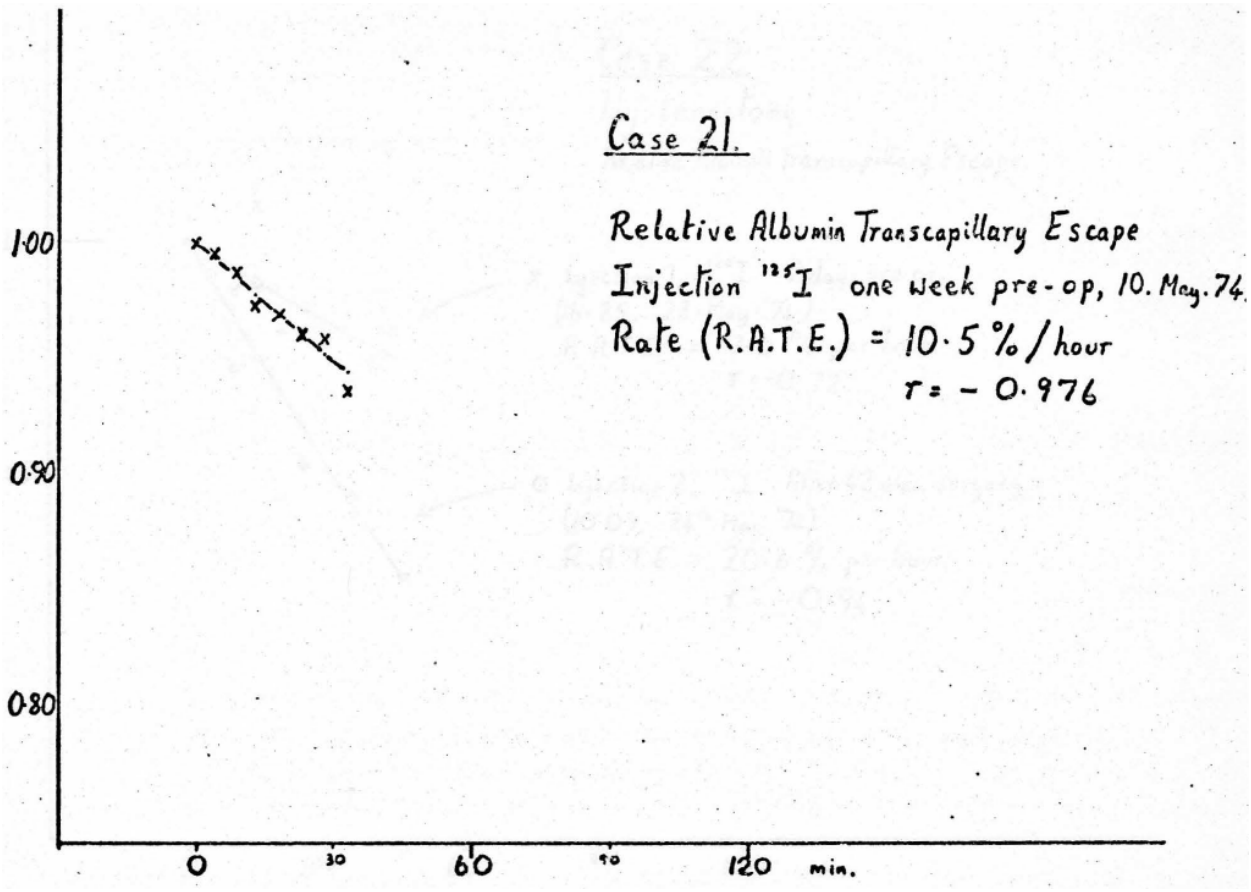
Mrs. V.T. Age 59. Anaesthesia commenced immediately following first series of samples taken.

I.V. Fluids. Lactated Ringer's solution, less than 100 ml. during the first measurement. After 53 minutes of anaesthesia:

Lactated Ringer's solution 500 ml.

Then during second measurement, Lactated Ringer's 150 ml.

Both graphs derived by regressing log (radio-iodine activity per gram/ radio chromium activity) against time. ^{51}Cr was included in first injection. See Method 9 a and b.

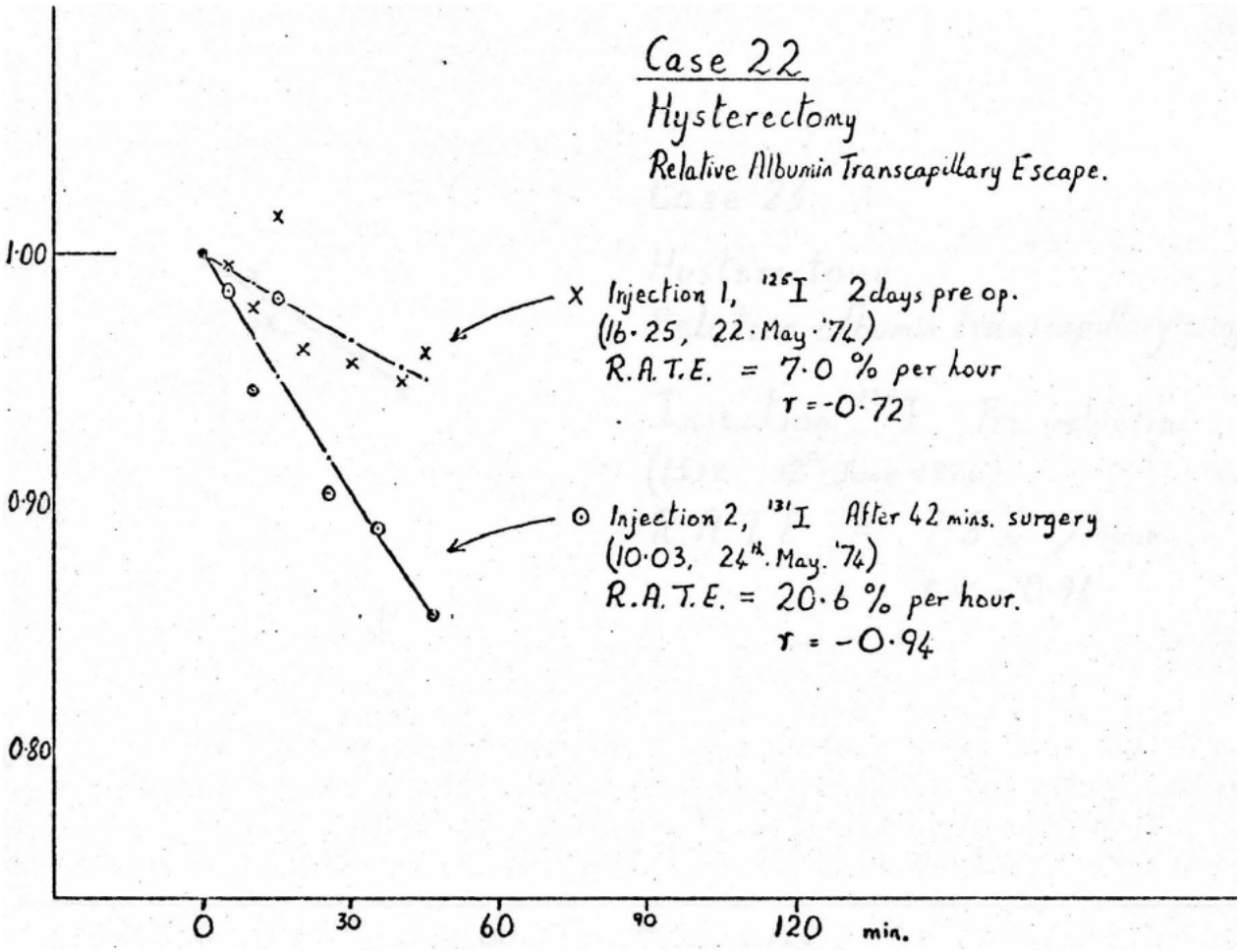


Mrs. B.J. Ago 73.

I.V. Fluid. Lactated Ringer's solution 75 ml. given prior to measurement, 75 ml. given during measurement.

Graph derived by regressing log (^{125}1 activity / ^{51}Cr activity) against time (see Method 9a),

This patient was selected because she was admitted for abdomino-perineal resection of rectum. In the absence of evidence of malignancy, no major surgery was performed.

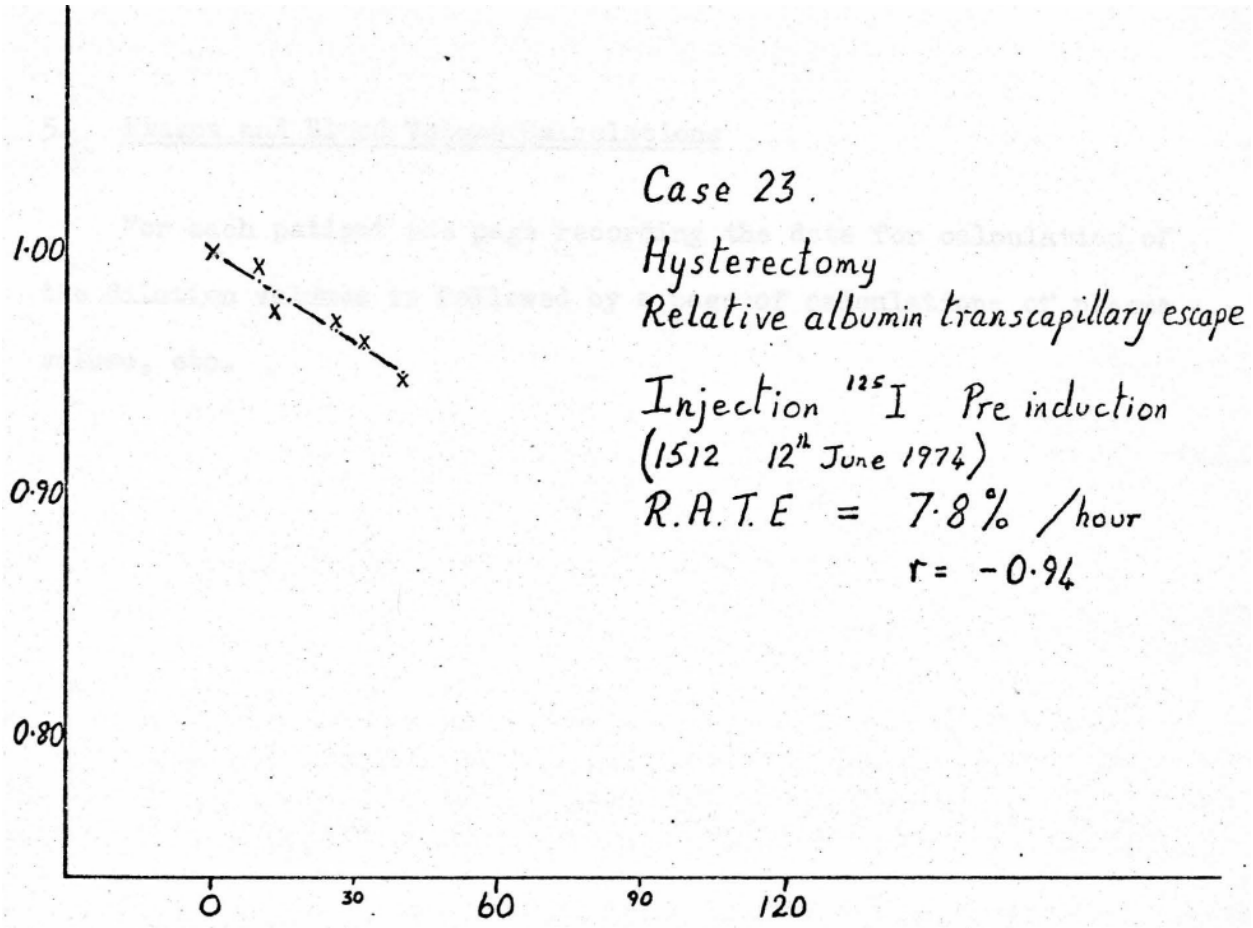


Mrs. R.M. Age 43.

I.V. Fluids. (22nd May 1974) Ringer's Lactate 200 ml. during measurement.

24th May 1974. See D.E.A.R.T.H. graph for this patient. Measurement started after 48 minutes of surgery. During measurement 50 ml. of Lactated Ringer's given.

Both graphs derived by regressing log (radio-iodine/radio-chromium) against time. ^{51}Cr was included in the first injection. See Method 9 a and b.



Mrs. P.E. Age 44.

I.V. Fluids (12th June 1974). Lactated Ringer's solution 75 ml. during measurement.

Graph derived by regressing log (^{125}I / ^{51}Cr) against time. See Method 9a.

The necessity to give rapid intravenous infusion precluded comparable measurement during surgery.

5. ~~Plasm~~ Plasma and Blood Volume Calculations

For each patient the page recording the data for calculation of the dilution volumes is followed by a page of calculations of plasma volume, etc.

Blood volume calculations. Patient Number: 20

Dilution volume = (Cts/gram Standard / Cts/g.pt y intcpt) X (Wt. inj. in pt. / Wt. add to stnd.) X Volume of stnd.

(10090 / 9679) X (5.003 / 0.529) X 500

= 4950 ml. Haematocrit = 41.87% (0.4187)

Isotope: ^{125}I Time: 1530 Specimen: 19

(47200 / 60500) X (5.064 / 0.444) X 500

= 4440 ml. Haematocrit = 0.42

Isotope: Time: Specimen:

(/) X (/) X

= ml. Haematocrit =

Patient 20. Mrs. V.T.

Blood volume calculations continued.

^{125}I / 51cr mixture injected at 1412, 16th April 1974.

^{131}I injected at 1530, 16th April 1974.

Activities per gram in patient calculated using log regression program C.

^{125}I activity per gram at t = 0 9679

^{131}I activity per gram at t = 0 60500

1412

Plasma volume = 4950 x 0.5813 = 2880

1530

Plasma volume = 4440 x 0.580 = 2580

Fall in plasma volume 300 ml.

Blood. volume calculations. Patient Number: 21

Isotope: ^{125}I Time: 1335. 10th May '74 Specimen: 3

Dilution volume = Cts/gram Standard 288630 / Cts/g.pt y intcpt 360822 X Wt. inj. in pt. 8.809 / Wt. add to stnd. 1.670 X Volume of stnd. 1000

= 4219 ml. Haematocrit = 0.38

Isotope: ^{51}Cr Time: 1335. 10th May '74 Specimen: 3

Dilution volume = Cts/gram Standard 167411 / Cts/g.pt y intcpt 259672 X Wt. inj. in pt. 8.809 / Wt.add to stnd. 1.670 X Volume of stnd. 1000

= 3401 ml. Haematocrit =

Isotope: Time: Specimen:

Dilution volume = Cts/gram Standard ____ / Cts/g.pt y intcpt ____ X Wt. inj. in pt. ____ / Wt. add to stnd. ____ X Volume of stnd. ____

= ____ ml. Haematocrit =

Patient 21. Mrs. B.J.

Blood volume calculations continued.

^{125}I / ^{51}Cr mixture injected at 1335, 10th May 1974. See R.A.T.E. graph.

Activity per gram in patient at time of injection calculated using log regression program C:

^{51}Cr activity per gram at t = 0	259672
^{125}I activity per gram at t = 0	360822

Red cell volume = 3441 x 0.38	=	1292
Plasma volume = 4219 x (1-0.38)	=	2610
Therefore blood volume	=	3902
Whole body haematocrit = 1292/3902	=	0.331

Blood volume calculations. Patient Number: 22

Isotope: ^{125}I Time: 1625, 22 May 74 Specimen: 4

$$\text{Dilution volume} = \frac{\text{Cts/gram Standard } 91364}{\text{Cts/g.pt y intcpt } 63232} \times \frac{\text{Wt. inj. in pt. } 10.186}{\text{Wt. add to stnd. } 2.924} \times \text{Volume of stnd } 1000$$

= 5030 ml. Haematocrit = 0.40

Isotope: ^{51}Cr Time: 1625, 22 May 74 Specimen: 4

$$\text{Dilution volume} = \frac{\text{Cts/gram Standard } 11994}{\text{Cts/g.pt y intcpt } 9975} \times \frac{\text{Wt. inj. in pt. } 10.186}{\text{Wt.add to stnd. } 2.924} \times \text{Volume of stnd } 1000$$

= 4180 ml. Haematocrit = 0.40

Isotope: ^{131}I Time: 10.03 24 May 74 Specimen: 3

$$\text{Dilution volume} = \frac{\text{Cts/gram Standard } 13234}{\text{Cts/g.pt y intcpt } 12541} \times \frac{\text{Wt. inj. in pt. } 7.830}{\text{Wt. add to stnd. } 1.950} \times \text{Volume of stnd } 1000$$

= 4240 ml. Haematocrit = 0.41

Patient 22. Mrs. R.M.

Blood volume calculations continued.

^{125}I / ^{51}Cr mixture injected at 1625, 22nd. May 1974. See R.A.T.E. graph.

^{131}I injected at 1003, 24th May 1974. See R.A.T.E. graph. Activities per gram in patient calculated using log regression program C.

^{125}I activity per gram at t = 0 63232

^{51}Cr activity per gram at t = 0 9975

^{131}I activity per gram at t = 0 12541

22nd May 1974. Red cell volume = 4180 x 0.40 = 1672 ml.

Plasma volume = 5030 x 0.60 = 3018 ml.

Therefore blood volume = 4690 ml.

Whole body haematocrit = 1672/4690 = 0.357

24th May 1974 after 350 ml. blood loss (approx. 225 ml. plasma).

Plasma volume = 4240 x 0.59 = 224.2 ml.,

i.e. plasma volume loss = 766 ml.

Blood volume calculations. Patient Number: 23a

Isotope: ^{125}I Time: 1512. 12th June 74 Specimen: 4

Dilution volume = (Cts/gram Standard 40325 / Cts/g.pt y intcpt 35877) X (Wt. inj. in pt. 5.355 / Wt. add to stnd. 1.335) X Volume of stnd. 1000

= 4509 ml. Haematocrit = 0.3712

Isotope: ^{51}Cr Time: 1512 12th June 74 Specimen: 4

Dilution volume = (Cts/gram Standard 46741 / Cts/g.pt y intcpt 49355) X (Wt. inj. in pt. 5.355 / Wt.add to stnd. 1.335) X Volume of stnd. 1000

= 3799 ml. Haematocrit = 0.3713

Isotope: Time: Specimen:

Dilution volume = (Cts/gram Standard / Cts/g.pt y intcpt) X (Wt. inj. in pt. / Wt. add to stnd.) X Volume of stnd.

= ml. Haematocrit =

Blood volume calculations. Patient Number: 23b

Isotope: ^{131}I Time: 1013 14th June 74 Specimen: 5

Dilution volume = (Cts/gram Standard 29468 / Cts/g.pt y intcpt 24129) X (Wt. inj. in pt. 5.878 / Wt. add to stnd. 1.894) X Volume of stnd. 1000

= 3790 ml. Haematocrit = 0.3883

Isotope: ^{51}Cr Time: 1013 14th June 74 Specimen: 5

Dilution volume = (Cts/gram Standard 15600 / Cts/g.pt y intcpt 16389) X (Wt. inj. in pt. 5.878 / Wt.add to stnd. 1.894) X Volume of stnd. 1000

= 2954 ml. Haematocrit = 0.3883

Isotope: Time: Specimen:

Dilution volume = (Cts/gram Standard / Cts/g.pt y intcpt) X (Wt. inj. in pt. / Wt. add to stnd.) X Volume of stnd.

= ml. Haematocrit =

Patient 23. Mrs. P.E.

Blood volume calculations (see previous page as well).

^{125}I / ^{51}Cr mixture injected at 1512, 12th June 1974.

^{131}I / ^{51}Cr mixture injected at 1013, 14th June 1974.

Activities per gram in patient calculated using log regression program C:

^{125}I activity per gram at t = 0	35877
^{51}Cr activity per gram at t = 0 (1512)	49355
^{131}I activity per gram at t = 0	24129
^{51}Cr activity per gram at t = 0 (1013)	29679
^{51}Cr activity before injection (1008)	13290
Increased activity caused by injection	16389

12th June 1974.	Red cell volume = 3799 x .3713	=	1411 ml.
	Plasma volume = 4509 X .6287	=	2835 ml.
	Therefore blood volume	=	4246 ml.
	Whole body haematocrit = 1411/4246	=	0.332
14th June 1974.	Red cell volume = 2954 x .3883	=	1147 ml.
	Plasma volume = 3790 x .6117	=	2318 ml.
	Therefore blood volume	=	3465 ml.
	Whole body haematocrit = 1147/3465	=	0.331
	Therefore red cell loss = 1411 - 1147	=	264 ml.
	and plasma loss = 2835 - 2318	=	513 ml.

6. Effect of Haemodilution on Red Cells

The effect of dilution is to reduce the haematocrit and the activity per gram of both red cell and albumin. However, if the haemodilution causes the red cell to swell or contract then there will be a discrepancy between the relative change in haematocrit and the activity. As the rate at which ^{51}Cr leaves the red cell is negligible over the duration of these measurements, the red cell activity is a measure of the number of red cells present. When the haematocrit is divided by the relative red cell activity it provides a measure of the amount by which the cells have swollen.

As one possible cause of such swelling could be the fall in albumin level, the following two pages report the results of experiments testing a linear relationship between red cell swelling and falling albumin level.

6. Effect of Haemodilution on Red Cells - Patient 7

1 Time	2(x) ^{125}I activity Normalized	3 Haematocrit %	4 ^{51}Cr activity normalized	5(y) Haematocrit/ ^{51}Cr activity
1351	1.000000	37.65	1.000000	37.6500
1353	0.985423	37.65	0.978797	38.4655
1355	0.993910	37.05	0.989319	37.4500
1357	1.016885	37.80	1.019525	37.0760
1359	0.990138	36.60	1.012565	36.1458
1401	0.995928	37.00	0.997999	37.0741
1403	0.994633	37.00	O.995660	37.1612
1430	0.960827	35.25	0.949053	37.1422
1432	0.957390	34.75	0.941633	36.9039
1452	0.955319	35.00	0.954925	36.6520
1454	0.954376	35.25	0.959970	36.7198
1508	0.932088	35.00	0.938373	37.2986
1510	0.933172	35.00	0.940841	37.2007
1532	0.927620	34.55	0.943806	36.6070
1534	0.927065	34.85	0.928441	37.5360
1546	0.856043	31.80	0.842013	37·7666
1548	0.870919	31.75	0.846979	37.4861
1611	0.809345	30.25	0.802387	37.7000
1613	0.763457	29.85	0.750620	39.7671
1633	0.856378	34.50	0.893298	38.6209
1634	0.848887	34.00	0.882640	38.5208
1635	0.851434	34.50	0.884014	39.0265

Linear regression: y = 45.36 - 8.44x. This suggests that if the albumin activity fell to zero the red cells might increase in volume in proportion to 36.92 to 45.36 (correlation coefficient 0.6837).

6. Effect of Haemodilution on Red Cells - Patient 10

1 Time	2(x) ^{125}I activity Normalized	3 Haematocrit %	4 ^{51}Cr activity normalized	5(y) Haematocrit/ ^{51}Cr activity
1415	0.999517	38.5	1.002801	38.3924
1415	1.001277	38.0	1.000830	37.9684
1415	0.999252	38.0	O.996367	38.1385
1445	0.976067	37.5	0.963157	38.9344
1500	O.940181	37.3	0.925793	40.2897
1515	0.954232	36.5	0.938100	38.9084
1530	0.934975	36.2	0.946407	38.2499
1545	0.928492	36.2	0.927609	39.0250
1600	0.819603	32.6	0.802148	40.6408
1610	0.797045	31.4	0.774270	40.5543
1625	0.845271	33.5	0.813624	41.1738
1640	0.836498	32.6	0.815285	39.9860
1655	0.836209	33.0	0.823225	40.0862

Linear regression: y = 50.70 - 12.37x. This suggests that if the albumin activity fell to zero, the red cells might increase in volume in proportion to 38.33 to 50.70 (correlation coefficient 0.8589).

The regressions calculated on the previous pages indicate a significant correlation between the albumin concentration and the red cell volume. Therefore, in the range of albumin dilution encountered in the experiments described, an appreciable but small alteration in red cell volume might be encountered. In one patient (19) the haematocrit was employed to calculate the R.A.T.E. If the regressions calculated for patients 7 and 10 are representative, then the R.A.T.E. calculated by Method 9c might slightly exaggerate the rate of albumin loss. In this experiment the fall in albumin level was not measured. Even if it had been fairly high (e.g. 6% in the half-hour measurement period) the regressions calculated suggest that the red cell volume might have increased by 0.6 in 38 or about 2%. During one hour, therefore, this might have accounted for about 4% or about one-seventh of the measured change. No correction has been applied for this. Even if so great a correction were necessary, there would still be a marked rise in the R.A.T.E. during surgery in patient 19.

7. Review of D.E.A.R.T.H. graphs

a) Patients undergoing major surgery under general anesthesia

The principal results were the series of observations made in twelve patients (numbers 2, **3, 6, 7, 9, 14, 15, 16,** 17, 18, 22 and 23) who were subject to major surgery under general anaesthesia. In every case there ***was a*** Degree of Extravasation of Albumin Relative To Haemoglobin (D.E.A.R.T.H.) which exceeded three standard deviations. In eleven of the twelve cases the fall in relative ratio occurred before surgery was completed. The twelfth (patient 3) was the only patient to have a significant rise in the relative ratio; even in this patient this rise was confined to a single observation and was followed immediately by an even more significant fall. Of more than 80 observations made on the relative ratio during major surgery under general anaesthesia, this was the only reading obtained which rose above the 95% confidence limit of the initial value. In contrast, over 60 values were found below the 95% confidence limits and, even if the fall in level is measured from the first value obtained during surgery, 50 values are obtained which indicate a significant fall. Thus, even in cases where there had been some D.E.A.R.T.H. prior to the incision **(14, 15, 18** and 23), there was a significant further fall in the relative ratio during surgery.

For most of the patients the magnitude of the change was greatly in excess of three standard deviations (one standard deviation was approximately equal to a change of 1%). D.E.A.R.T.H. in excess of 10% was observed in four patients. Three of these (6, **14** and 18) were undergoing hysterectomy and the fourth (9), who had an abdomino-perineal resection of rectum, showed a D.E.A.R.T.H. of 13%. These changes were recorded between 80 and 110 minutes after the surgical incision.

b) Patients undergoing other procedures

Six patients (numbers 5, 10, 12, 13, 18 and 23) were examined under other conditions. Patient 18, who showed a D.E.A.R.T.H. of over 10% during surgery, end patient 13 were both observed on the day preceding their surgery. Measurements were also made on patient 13 and on patient 10 while undergoing hysterectomy under epidural anaesthetic. Patients 5 and 12 underwent stapedectomy under general anaesthesia and patient 21 had a sigmoidoscopy without anaesthesia.

Measurements were made on patients 13 and 18 during control periods of 75 minutes and 90 minutes respectively. There was no continuing change, although it is remarkable that in both series of observations made on patient 18 there was a significant fall in the relative ratio immediately following the venepuncture. In neither of the patients having stapedectomy was the surgery associated with a continuing change, although there had been an earlier fall in patient 12 during the period following the venepuncture. However, in both these patients, the period of emergence was associated with a fall in the relative ratio and this was most marked in patient 12 where there was a fall of 4.6% in the ten minutes with a further fall of 1.4% in the next eleven minutes.

Neither of the patients undergoing hysterectomy under epidural anaesthesia showed a fall in relative ratio during about two hours following the incision. However, when the effects of the epidural anaesthetic began to diminish in patient 13, there was an abrupt and significant fall of 3.4% in 15 minutes. In patient 10 there was a sustained rise with four consecutive values showing a rise of more than two standard deviations. One of these values showed a rise of more than three standard deviations (an increase of 3.9%).

Patient 21 was observed only for the period of her sigmoidoscopy. There were no significant changes but the period of observation was only 21 minutes.

c) Patients prior to surgery

In addition to the above observations, two or more measurements were also made during the pre-operative period in patients 3, 5, 6, 7, 9, 14, 15, 16, 22 and 23. Periods of observation ranged from only a few minutes to about 14 hours, with six periods exceeding an hour. In these six longer periods four patients showed no significant change (3, 5, 6 and 22), while the fall in the other two just exceeded the 95% confidence limits (16 and 23). It was in another patient (9) that the highest pre-operative value for D.E.A.R.T.H. was observed (3.2%), but this was followed by a return to an almost normal value before the change during surgery.

d) Patients showing sudden changes in Relative Ratio

In many patients there was a sudden fall in relative ratio. Most commonly this was a fall within a few minutes of the incision for major surgery (patients 6, 9, 15, 16, 17, 18, 22 and 23), although in patient 17 there was no detectable change during the three-quarters of an hour of anaesthesia and minor surgery which preceded the cholecystectomy incision. In both days in patient 18 there was a similar significant fall in the first fifteen minutes following venepuncture, and in patients 5, 7 and 12 there was a significant fall associated with emergence from anaesthesia. In no patient emerging from general anaesthesia was there a rise in relative ratio. A fall occurred during emergence in other patients (2, 3, 6, 14, 15 and 16), but this was difficult to distinguish from the fall occurring already,

although it appeared to be accelerated if anything. In three patients (13, 14. and 15) the period of the attempted epidural was associated with a significant fall in the relative ratio. This was most marked in the two unsuccessful attempts (patients 14 and 15) which were prolonged and caused the patient some discomfort. A D.E.A.R.T.H. of 6.4% and 4.4% respectively was recorded during the attempt.

Between pairs of consecutive readings there were only three significant increases in relative ratio in the entire series of observations. These occurred in patients 6, 9 and 15. The changes did not represent continuing trends and appeared to have no common associations. The only patient in which there was a continuing rise in relative ratio was in patient 10, described above, where under epidural anaesthesia the relative ratio rose by 3.9%, the highest rise in ratio recorded in the series.

8. Review of R.A.T.E. results

Towards the and of the series of D.E.A.R.T.H. results, the method for measuring R.A.T.E. was devised to discover whether it was possible to measure rates of albumin extravasation high enough to account for the D.E.A.R.T.H. In patients 19, 20, 21, 22 and 23 the R.A.T.E. per hour measured prior to surgery was 10.4$, 9.9%, 10.9%, 74% and 7.0% with a mean of 9.1%; per hour. Measurements of R.A.T.E. taken during an abdomino-perineal resection (19), a cholecystectomy (20) and a hysterectomy (22) were 274%, 15.3% and 20.6%, with a mean of 21.0% per hour. The greatest increases were observed in patients 19 and 22 where the R.A.T.E. rose by 16.65 and 13.0% respectively.

9. Review of blood volume findings

The introduction of R.A.T.E. measurements provided data which made it possible to study blood volumes, plasma volumes and whole body haematocrits.

The calculated plasma volume deficit, blood loss measured (by weight) and volume of Lactated Ringer's solution infused by the time of the second estimate are shown in the following table:

Patient number	Calculated plasma deficit (ml)	Measured blood loss (ml)	Lactated Ringer's infused (ml)
20	300	200	500
22	766	350	400
23	513	300	300

In these patients it is possible to correlate the different results. In patient 22 and 23 D.E.A.R.T.H. was measured and there was a significant extravasation of albumin (7.6% and 7.2% after 40 minutes surgery). In these two patients and in patient 20 there was a considerable fall in plasma volume, a fall which exceeded the measured loss of blood despite the patient being given intravenous fluid. The rate of loss of albumin from the circulation rose from 9.9% to 15.3% in patient 20 and from 7.0% to 20.6% in patient 22.

The whole body haematocrit was calculated in patients 21 and 22 and twice in patient 23. The discrepancy found between peripheral venous haematocrit (blood taken from subclavian vein, innominate vein or superior vena cava) and the calculated whole body haematocrit is shown in the following table:

Patient~ number	Peripheral venous haematocrit	whole body haematocrit
21	0.380	0.331
22	0.400	0.357
23a	0.371	0.332
23b	0.388	0.331

Table showing peripheral venous and whole body haematocrits.

10. Summary. of results

Twelve patients undergoing major surgery under general anaesthesia all had significant D.E.A.R.T.H., although this was delayed in one patient until the end of surgery. Enhanced R.A.T.E. and a disproportionately large fall in the plasma volume were also observed during major surgery under general anaesthesia.

A sudden increase in the D.E.A.R.T.H. was observed following incision and following venepuncture, during attempts to give an epidural anaesthetic, during emergence from general anaesthesia and on one occasion where the epidural anaesthetic became inadequate.

Periods of observation during minor surgery or during control periods were not associated with a marked D.E.A.R.T.H.

D I S C U S S I 0 N

The measurement during surgery of changes in capillary permeability to albumin is complicated partly by the effects of surgery and partly by the effects of essential associated therapy. However, by employing for reference purposes a particle larger than the albumin molecule which does not escape from the circulation, then the loss of albumin particles due to capillary permeability can be measured; dilution and haemorrhage affect both the particles similarly whereas albumin extravasation does not. The larger the particle, the more satisfactory should be its properties as a reference, and the isotope-labelled red cell was chosen on this basis.

Unfortunately, when a ratio is employed for measurement, it makes no distinction between a rise in one variable and a fall in the other. In addition, there may be uneven distribution of the two particles in the circulation. The discrepancy observed between the peripheral haematocrit and the whole body haematocrit indicates that somewhere in the body there is blood with a very low haematocrit. For example, capillary blood may contain very few cells. These factors require consideration before any results can be interpreted.

The major finding is a relative fall in the level of albumin in the blood (D.E.A.R.T.H.). The possible explanations for this would be albumin extravasation or release into the circulation of stored red cells. A store of red cells is possible, and in some species the spleen has a significant capacity. However, in man, the spleen is normally regarded as an insignificant store, and the evidence obtained in this series of experiments tends to confirm this. When the red cell/albumin mixture was injected prior to the R.A.T.E. measurements there was no evidence of any initial decline in red cell

radioactivity to indicate uptake of cells into a store. Moreover the low whole body haematocrit already indicates that there must be a surprisingly low haematocrit in, for example, the capillaries. If, in addition, a red cell store were postulated, it would require in balance an even greater red cell dilution elsewhere. The possibility cannot be excluded that injected radioactive red cells are stored and then released during surgery, but it must be regarded as an improbable explanation of the findings and one which is not supported by the R.A.T.E. and plasma volume results.

Another possible explanation for the observed fall in the ratio would be provided by variation in the discrepancy between peripheral haematocrit and whole body haematocrit. Blood taken from a vein provides an over-estimation of the whole body haematocrit, indicating a smaller proportion of plasma than is in fact present in the circulation. If this discrepancy became worse, then peripheral blood would indicate a rising haematocrit when in fact there might be no plasma loss and no change in whole body haematocrit. However, this explanation is also inconsistent with the demonstrated falls in plasma volume, and it is therefore unlikely that an increase in the disparity between peripheral and whole body haematocrits is the explanation for the fall in relative ratio.

Rapid intravenous infusion might also be expected to produce redistribution of fluid with a possible effect on the discrepancy between whole body and peripheral venous haematocrits. There is no clear evidence in this series of patients of a consistent change following infusion. Only in patients 7, 10 and 18 was there marked haemodilution from infusion. The slight rise in the relative ratio which appears in patients 7 and 10 is not found in patient 18, although the degree of dilution was greater. However Dextran 70 was included in patient 18 and this could conceivably have had a

different effect. In none of these cases was there any indication that intravenous infusion of dextrose or lactated Ringer's solution might account for the fall in the relative ratio observed elsewhere in the series.

A steady loss of radio iodine from the albumin pool would also result in an apparent loss of albumin from the circulation. Degradation of R.I.H.S.A. with replacement by non-radio-active iodine occurs, but the rate of loss is slow and is to some extent balanced by the loss of radio chromium from the red cell. Observation in patient 13 confirms this. Although only three days had elapsed from the time of the injection of the isotopes, the overnight rate of loss of iodine radioactivity was down to about 12.2% in 23 hours, i.e. about 0.55% per hour. However, in the same period there was a decline in the radio chromium activity of 8.2% In consequence, the apparent change in relative ratio was only 4.4% in 23 hours, or less than 0.25 per hour. In the majority of patients there was an even longer time for equilibration, and in consequence the error from this source may be smaller rather than greater; the period normally required for equilibration has been shown to be about five days (Mouridsen and Faber, 1966).

Because it accords with the other results, the only tenable explanation to account for most of the observed change in the relative ratio is an extravasation of albumin. The normal rate of turnover of albumin between the vascular and extravascular compartments is reported to be about 5% per hour (Yoffey and Courtice, 1970; Parving and Gyntelberg, 1973). Somewhat higher resting values were obtained here but the values found during surgery were considerably higher still; the rate of albumin loss rose to double or even treble the resting rate.

There are several possible explanations of a high rate of albumin extravasation during surgery. As capillary blood may have a considerably lower haematocrit than the rest of the blood, then an apparent extravasation of albumin might be attributed to capillary haemorrhage. However, the rate of change in the relative ratio seems to be as high or higher late in surgery when haemorrhage would normally be controlled. Moreover, although the composition of capillary blood may differ markedly from blood in large vessels, the delivery of blood from a capillary has to depend on the composition of blood brought to it by the large vessels. The red cells may speed through the capillary and be present in a correspondingly low concentration, but the number which leave the capillary related to a given quantity of plasma should be the same as in the large vessel supplying the blood. While it might be conceivable that some channels could carry more plasma and fewer cells it is difficult to believe that such vessels might bleed selectively while others continue to circulate blood rich in red cells.

A local increase in capillary permeability associated with the trauma and mediated by the liberation of histamine and/or serotonin is a more probable explanation of localised albumin extravasation. The quantity of albumin which would have to be deposited at the site of surgery to account for the measured D.E.A.R.T.H. in this series would be about 4 - 16 grams (i.e. about 3 - 13% of the intravascular pool). Measurements of extravasation (R.A.T.E.) appeared to indicate an even more rapid loss of albumin, but this figure includes albumin turnover as well, and the normal losses which are occurring elsewhere in the body are balanced by lymphatic return.

Some of the observations made, however, are not readily explained by local traumatic extravasation of albumin. The falls in relative ratio encountered at the end of surgery, in association with

the epidural attempts and as an epidural wore off appear inconsistent with the magnitude of the trauma involved. Rapid local deposition of several grams of albumin would be required in addition to any local haemorrhage. Similar considerations apply to the fall in relative ratio observed following venepuncture in both sets of observations made in patient 18. The venepuncture was carried out without difficulty and caused no apparent distress, and yet there was a significant fall in the relative ratio on both occasions. A redistribution of fluid in response to pain or in response to getting into bed and assuming the supine position might account for a change in the concentration of albumin or of haemoglobin. However it would only produce simultaneous changes in opposite directions if the redistribution caused an increase in the discrepancy between peripheral and whole body haematocrits. Even the changes found in patient 18 would require a substantial enhancement of this discrepancy (e.g. approximately 50%). Although such a change cannot be ruled out, it is not an entirely satisfactory explanation either for this or for similar increases in relative ratio.

An alternative explanation might be that changes in capillary permeability might be due to release of a vaso-active substance such as kallikrein and that as a result there might be widespread loss of albumin from the circulation. Experiments in dogs (Macfarlane et al., 1973) demonstrate that kallikrein infusion increases capillary permeability and also that this effect is more pronounced in the presence of surgery (nephrectomy). The increases recorded in these dogs were of similar magnitude to those recorded in these experiments.

Diminished synthesis and diminished lymphatic return might also contribute to an overall depression of the relative ratio. However, synthesis rates are too low to account for any major change and

lymphatic return almost certainly persists; the very high R.A.T.E. measured would cause a very much greater D.E.A.R.T.H. if there were not a continuing lymphatic return to compensate to some extent for the extravasation.

In contrast to the various possibilities discussed which might account for the sudden falls in relative ratio which were encountered, it is difficult to foresee circumstances causing the reverse. Sudden liberation of albumin or abrupt removal of red cells from the circulation are both hard to envisage. In the mass of observations made, a statistically significant ($P = 0.05$) rise between consecutive observations occurred no more frequently than chance alone would produce. There were no common associations and there seems no reason to postulate the existence of any underlying mechanism.

A mechanism does exist however which might account for a slow, steady rise in the intravascular albumin. Diminution in capillary permeability has been demonstrated to accompany plasmapheresis in rats (Wraight, 1974). If capillary permeability did fall during a period of observation in these experiments then lymphatic return might be expected to raise the relative ratio by up to a maximum of about 5% per hour. Only in patient 10 (hysterectomy under epidural) was any progressive rise seen. As this was one of the patients where a considerable volume of intravenous fluid was infused, it is inappropriate to draw any conclusion as to whether this provides evidence of reduced capillary permeability in this series of patients.

Whatever the mechanisms may be which underlie the D.E.A.R.T.H. observed in patients undergoing major surgery under general anaesthesia, the changes are significant statistically and also of practical significance in the patient's management. A surgical

patient is known to be normally somewhat dehydrated by being deprived of oral fluids pre-operatively, and in addition his blood loss should be known (although, as in these patients, any measurements of loss tends to be an underestimate). The correction which is made for dehydration and blood loss depends on the magnitude of the blood loss and on the preference of the surgeon and the anaesthetist. A cautious approach to the use of intravenous fluid therapy is based on a fear of over-infusion and is justified by a reassuring and widespread belief that surgery is accompanied by a compensatory redistribution of fluid which helps to maintain the blood volume by expanding the plasma. If such compensation occurs there is no evidence of it in this series of patients. Indeed, it is the reverse which appears to occur, and it would seem that a significant loss of plasma should be added to any other estimated or measured fluid losses. When this is coupled with the reluctance to expose patients unnecessarily to the hazards of blood transfusion and with the tendency to underestimate blood loss, it means that all patients undergoing major surgery are likely to finish with a significant reduction in blood volume.

The significance of this reduction can best be appreciated by considering the effects of treating blood loss with intravenous saline or with lactated Ringer's solution. A patient undergoing hysterectomy with an estimated blood loss of 200 ml. might receive during surgery 500 ml. of dextrose 95 and 500 ml. of lactated Ringer's solution. The replacement of a 200 ml. blood loss with 1000 ml. of intravenous fluid might be regarded as sufficient to maintain the volume of the circulating blood. However, the patient has probably lost in addition about 10% of her circulating albumin, equivalent to about 300 ml. of plasma. Thus the overall loss of blood and plasma is about 500 ml. As the saline and dextrose given will be distributed throughout the extra-vascular space and total

body water respectively, they will only account for an increase in blood volume of about 200 ml. There remains a blood volume reduction of 300 ml., and if it were desired to correct this using saline (or lactated Ringer's solution) a further 1500 ml. would be required. Thus to maintain the blood volume in a patient who lost 200 ml. of blood during a hysterectomy, about 2.5 litres of intravenous fluid would be required. This assumes that the estimate of blood loss is correct; it makes no correction for the preoperative dehydration and makes no allowance for continuing loss of albumin at the site of surgery. In view of these assumptions, 2.5 litres is probably a conservative figure, and it is likely that even with such treatment the patient would finish with a diminished blood volume.

Maintenance of the blood volume with electrolyte solution is not however free from complications. Hutchin et al. (1969) showed that although a diuresis could be maintained by administration of balanced salt solutions, this was accompanied by severe pulmonary congestion. In addition, Moss (1969) showed that as blood loss progresses and is made good with balanced salt solution$_s$ so the distribution will increasingly favour the extravascular compartment of the extracellular space; less and less of any salt solution infused will remain in the circulation.

Because albumin extravasation is equivalent to plasma loss and because of the disadvantages of maintaining blood volume with salt solution, the results described in this thesis lend support to the use during surgery of plasma expanders such as dextran, plasma, blood and, if sufficient is ever available, human serum albumin.

CONCLUSION

The measurement methods developed and described in this thesis allow measurements to be made of the ratio between albumin and haemoglobin in the circumstances obtaining during surgery. From these are derived measurements of change in the quantity of intravascular albumin (D.E.A.R.T.H.), measurements of the albumin extravasation (R.A.T.E.) and measurements of blood volume and plasma volume.

The results obtained by these methods suggest that relative to haemoglobin there is during surgery a loss of intravascular albumin and a raised rate of albumin extravasation, and that there is a disproportionate fall in plasma volume. The findings indicate that surgery is accompanied by an additional fall in blood volume over and above the reduction expected from dehydration and measured blood loss. The size of this loss must vary from patient to patient, but in a hysterectomy might be 300 ml. before completion of surgery, and could be considerably larger in more extensive surgery.

The implication of this additional loss of blood volume is that the common custom of infusing the surgical patient with saline and dextrose is unlikely to maintain the normal blood volume and that consideration should be given to the more widespread use of a plasma volume expander, even in the absence of significant blood loss.

DEFINITIONS

Capillary permeability to albumin refers to the escape of albumin from the circulation. Even though the site of this loss may be the venules and small veins rather than the capillaries, the term "capillary permeability" is in widespread use and has been retained.

D.E.A.R.T.H. Degree of Extravasation of Albumin Relative To Haemoglobin measures the loss of intravascular albumin although the blood may be subject to simultaneous dilution or concentration. D.E.A.R.T.H. is the fall in concentration of albumin relative to the haemoglobin level. It is measured by the change in the relative ratio (see below) some days following injection of the isotopes to allow equilibration of intra- and extravascular albumin.

Oncotic pressure is the osmotic pressure due to larger molecules, such as those of protein, which are unable to pass through semi-permeable membranes that allow free passage to salt and water. Oncotic pressure is proportional to the number of molecules present. In blood the relatively high concentration and the small molecular weight of albumin means that the greater part of the oncotic pressure is attributable to albumin.

R.A.T.E. The Relative Albumin Transcapillary Escape measures the rate at which intravascular albumin escapes from the circulation although the blood may be subject to simultaneous dilution or concentration. R.A.T.E. is the fall per hour in the albumin level. It is measured by the relative change in radioactivities during the period immediately following injection of R.I.H.S.A. and ^{51}Cr-haemoglobin.

Relative ratio. The ratio between R.I.H.S.A. and ^{51}Cr relative to the ratio obtained in the initial sample of the series. Thus the initial sample of blood taken has a relative ratio of 1.0 and a falling relative ratio indicates a relative loss of albumin

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